ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 42 SUPPLEMENTUM 147
FROM THE NOBEL INSTITUTE FOR NEUROPHYSIOLOGY,
KAROLINSKA INSTITUTET, STOCKHOLM 60, SWEDEN

SHORT TERM REACTIONS IN THE THYROID GLAND

revealed by continuous measurement of blood flow, rate of uptake of radioactive iodine and rate of release of labelled hormones

BY

ULF SÖDERBERG

STOCKHOLM 1958

VICTOR PETTERSONS BOKINDUSTRIAKTIEBOLAG
STOCKHOLM 1958

CONTENTS

	Page
INTRODUCTION	5
METHODS	7
Anatomy and vascular supply of the thyroid	8
Nerve supply	10
Recording of blood flow	10
The collection of blood samples for the assay of radioactive substance	12
Radioactive isotopes of iodine	14
Brain stimulation	15
Histological control	15
RESULTS	
Thyroid blood flow	16
Sympathetic nerves	17
Superior laryngeal nerve	19
The recurrent nerve	19
Reflexes from the vagus	20
'The arousal reaction'	24
Hypothalamic heating	27
Adrenaline and noradrenaline	28
Acetylcholine	30
Serotonin (5-hydroxytryptamine)	30
Reflex discharge of catechols from the adrenal medulla	31
Thyrotropic hormone (TSH)	33
Antidiuretic hormone (ADH)	35
Adrenal cortical hormones	37
Adrenocorticotropic hormone (ACTH)	37
Anaesthetics	37
Drugs inhibiting thyroid activity	39
The uptake of inorganic iodine from the blood	41
The A—V difference of radioiodine	42
Anaesthetics	53
Thiouracil and mercaptobenzimidazol	54

Discharge of labelled iodine from the thyroid 55
The release of labelled hormone 59
Thyroid stimulating hormone (TSH) 61
Influence of thyroid blood flow 62
Sympathetic stimulation 62
Superior laryngeal nerve 63
Reflexes 64
Adrenaline and noradrenaline 67
Acetylcholine 71
Serotonin 76
Antidiuretic hormone 77
Circulatory failure and oxygen lack 77
Anaesthetics 77
Musculus levator glandulae thyroideae 78
The relation between uptake of radioiodine and rate of secretion of labelled hormone 79
Uptake of labelled phosphate by the gland 82

DISCUSSION
Sources of error 83
Anastomoses 84
Net fluid shifts 85
Changes in drop volume 85
Anaesthesia and operation 86
Maintenance of circulatory blood volume 86
Heparinization 86
Sedimentation of blood cells in the cannula 86
Distribution of isotopes 87
Radiation damage 88
Baroreceptor and 'volume receptor' influence 88
Uptake of iodine 90
Iodine clearance 91
Hormone secretion 92
Serotonin 94
Seasonal variations 94
The thyroid-pituitary relationship 95
Iodine shortage 98
Thyroid storm 99
Thyroid uptake of TSH 99
SUMMARY AND CONCLUSIONS 101
ACKNOWLEDGEMENTS 103
REFERENCES 104
APPENDIX 109

INTRODUCTION

At the beginning of this century the circulation of the thyroid gland was the subject of considerable physiological investigation which was prompted largely by two facts. The thyroid blood flow was the only measurable index of thyroid activity then known, and ligation of a part of the arterial supply to the gland was commonly used in the surgical treatment of toxic goiter. The vascular anatomy and physiology of the thyroid had therefore been extensively described (references in "Appendix" on p. 109) when the use of radioactive isotopes was introduced to the study of thyroid function (references in MEANS, 1948, and RIGGS, 1952). This modern technique made possible an analysis of thyroid activity based on repeated measurements of thyroid iodine levels in intact animals and man, and, in addition, another recent technique, partition chromatography, permitted the separation of the minute quantities of iodine compounds that could be extracted from the thyroid and from other tissues. Older physiological studies, dealing for the most part with the influence of sympathetic nerves and adrenaline (references in "Appendix"), were more or less forgotten, particularly when it was demonstrated that exophthalmus was not the result of sympathetic stimulation.

However, the indirect determination of iodine clearance, another recently introduced measure of thyroid activity, is one which is intimately related to thyroid blood flow. Studies of a few serial venous blood samples have been made to measure the efficiency with which iodide is removed from blood flowing through the gland. Likewise, samples of thyroid venous blood taken from large animals pretreated with radioactive iodine have been analysed for hormonal compounds (*cf.* POCHIN, 1950 and TAUROG, WHEAT and CHAIKOFF, 1955).

It was therefore considered of interest to devise a method which

would permit a continuous measure of the arteriovenous concentration difference both of iodide and of thyroid hormones. If this could be done by combining the older physiological technique of measuring venous outflow from the thyroid, introduced by OSSOKIN (1914), WATTS (1915) and GUNNING (1917) with the modern use of tracers for the assay of iodine in the blood, a very sensitive method of describing thyroid iodine metabolism would result. This would also yield valuable information for the study of compounds secreted by the thyroid gland, since it would be possible to know the exact moment at which samples must be drawn from the thyroid venous blood to permit accurate analysis.

The purpose of the present investigation was, then, to develop the method outlined above, and to study briefly some nervous and hormonal influences on thyroid activity.

The rabbit has been the experimental animal of choice since it was the subject of the fundamental researches of Dr. G. W. Harris and his colleagues, for it is their contributions to thyroid physiology which have provided a firm basis for the present investigation (for references see BROWN-GRANT, 1957 and HARRIS and WOODS, 1957). The rabbit, and the cat as well, though small in size, are particularly suited to this study because the technique of measuring venous outflow is favoured by low rates of flow; this outweighs the difficulties encountered in cannulating the small veins of these animals.

The results to be presented show that it is possible to record the thyroid arteriovenous iodine concentration difference and blood flow continuously for several hours with a high degree of accuracy and good temporal resolution. This technique has revealed, and permitted the measurement of changes in activity which are both rapid and frequent and so provides a useful index of vegetative activity in experimental animals.

METHODS

Experiments were performed on 19 cats and 66 rabbits (male and female) under light to moderate anaesthesia. Most of the animals were given pentobarbital ('Nembutal,' Abbott), 40 mg per kg body weight intravenously or intraperitoneally. Small doses of chloralose were often administered to the rabbits to make general vasomotor activity more stable than it usually is under Nembutal alone. Chloralose-urethane, one per cent chloralose in 10 to 20 per cent urethane, was also frequently given to rabbits. A few animals were anaesthetized with intermittent doses of thiopental ('Pentothal,' Abbott). *Encéphale isolé* and decerebrate preparations of cats were also used.

Operative wounds were as far as possible treated with local anaesthetic, Lidocaine ('Xylocaine,' Astra). Slightly hypertonic glucose solution was administered subcutaneously. Lamps or hot water bottles warmed the animals when necessary. Usually the animal lay on its back with the greater part of its head below heart level; this is of particular importance for rabbits under Nembutal anaesthesia. A head-holder was used only when stimulating electrodes were inserted in the brain.

The arterial blood pressure was measured by a strain gauge manometer (ELEMA) connected to the femoral artery by wide-bore polyethylene tubing. The manometer operated the mirror galvanometer of a photokymograph.

A tracheal cannula was inserted late in the experiment in most of the cases. In a few cats when very light anaesthesia was employed, no tracheal cannula was inserted to permit the recognition of undesirable decrease in anaesthetic depth. The tracheal cannula could be connected to valves permitting the selection of gases for inspiration. In a few experiments the respiration was recorded by means of a

spirometer, connected to the expiratory side of the respiratory valves, and registering the volume of each expiration with a mirror galvanometer. The spirometer was rapidly emptied every 30 sec by opening an electromagnetic valve between the spirometer and an evacuated bottle.

Anatomy and vascular supply of the thyroid

Anatomical features of importance for the present study are illustrated in Fig. 1 which shows the ventral *(A)* and dorsal (*B* and *C*) aspects of the laryngeal region of a rabbit. If the upper part of the trachea is exposed as for a usual tracheotomy the lateral lobes of the thyroid gland will be found tightly bound to the trachea immediately deep to the sterno-thyroid muscles, generally with a very thin, hardly recognizable *isthmus* between them on the ventral side of the trachea 5 to 10 mm below the larynx. The size and shape of the gland varies greatly from animal to animal, being larger in the autumn and winter than in the summer, at least in the rabbit. If the gland is dissected free and divided through the *isthmus* the halves are generally of the same size. Thus from 17 randomly chosen rabbits the left half had a weight of 99.2 $\pm$ 4.0 mg and the right half 98.5 $\pm$ 4.0 mg. The average difference between the two halves was 8.8 $\pm$ 3.4 per cent of the total weight. The gland is surrounded by a capsule which is very thin and elastic in the rabbit but thick and firm in the cat.

The main arterial supply is derived from the inferior laryngeal artery, one on each side. Sometimes one of these supplies most of the blood to both halves of the gland. The vessel is a long flexible branch of the common carotid artery. During the dissection of the region special care was taken not to touch the artery with instruments, for any trauma to it may cause a spastic constriction of long duration.

The thyroid veins (Fig. 1 *C*) communicate with veins from the larynx and adjacent parts of the trachea and oesophagus. Most of the venous blood appears to flow in a caudal direction in one vein on each side of the trachea but venous flow in the opposite direction may occur as the resistance of the veins going cranially *via* the larynx seems small, particularly in the rabbit. There is also generally a large communicating vein between the two halves of the gland on the

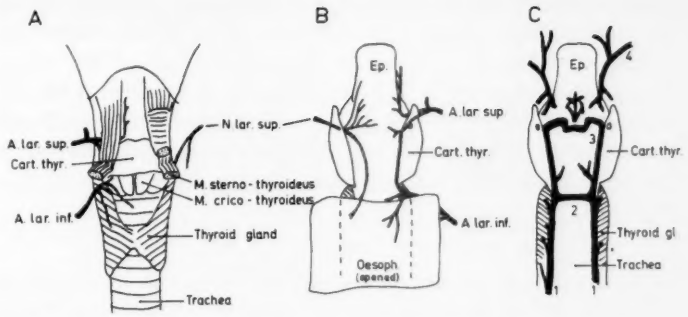

Fig. 1. The anatomy of the laryngeal region of the rabbit. (Schematic drawing partly after SCHNEIDER, 1867.) *A*, ventral aspect, *B* and *C*, dorsal aspect. In *A* arteries only on the left, nerves on the right, in *B* the opposite, in *C* veins on both sides. In *B*, part of the oesophagus is removed, the caudal portion which remains is opened, in *C* the entire oesophagus is removed. *Abbreviations: Ep.* epiglottis, *1*, the caudal veins, which follow the trachea down into the thorax, *2*, a communicant vein between the two halves of the gland, *3*, anastomoses to the larynx and to laryngeal veins (4) rostral to the thyroid, which open into the internal jugular veins.

dorsal side of the larynx (Fig. 1 *C: 2*) which is difficult to eliminate but can severely disturb the measurements. In a few cases a large vein has also been found following the inferior laryngeal artery to the internal jugular vein. In the cat the caudal veins frequently unite into a *vena thyroidea ima* on the ventral side of trachea. Sometimes, instead of the well-defined vessels mentioned above, a large number of small veins run along the trachea, making it impossible to record the venous drainage, since the resistance in the only vessel remaining for measurement must be low enough to avoid the development of oedema in the gland which otherwise appears rapidly when the venous pressure increases. Especially in the cat, where most of the venous outflow from the gland almost without exception follows the caudal veins, the vessel to be cannulated could not be clamped for more than a minute without the production of irreversible damage from pressure of the oedematous tissue inside the capsule. It was therefore necessary to cannulate the thyroid vein of the cat without clamping it between the gland and the hole cut in the vessel. Al-

though the loose capsule of the rabbit thyroid made it possible to use the conventional technique it was better to cannulate before the cranial veins had been ligated. A firm ligature around the whole larynx was then applied. This served at the same time to fix the tracheal cannula and to ligate the vessels between the thyroid and tissues cranial to it. The tracheal cannula was generally inserted in the mid-line between the two pairs of crico-thyroid muscles (see Fig. 1 *A*).

Nerve supply

The main nerve supply to the thyroid consists of relatively large fibres from the vagus running chiefly in the superior laryngeal nerve and the recurrent nerve, the former seen in Fig. 1 *A* and *B*, the latter following the caudal thyroid vein on each side of the trachea. Fibres from the cervical portion of the sympathetic trunk follow the arteries but also join both the superior laryngeal and the recurrent nerves.

Recording of blood flow

The rate of blood flow from the thyroid was measured by the conventional technique of recording the venous outflow which avoids clotting by complete heparinization (*cf.* LINDGREN, 1955). An open drop chamber was used, and the free end of the cannula was kept below the level of the gland in order to keep the venous pressure low. An electronic ordinate writer operating a mirror galvanometer was used for the registration of the time interval between the drops, schematically shown in Fig. 2. Each time interval is recorded as a steeply rising line whose length is directly proportional to the length of the interval and consequently indirectly proportional to the rate of flow. This method permits direct recording of the time at which each drop of blood falls from the cannula and the rate of flow on a very condensed time scale.

A similar technique was used for blood flow from other organs and for urine drops from a cannulated ureter. The rate of flow could also be calculated from the distance between drops that fell on the moving strip of filter paper described below.

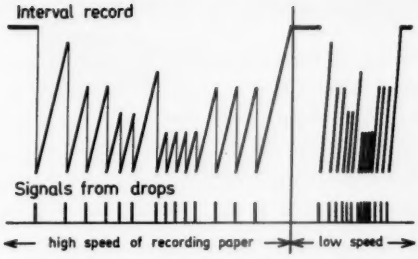

Fig. 2. Schematic drawing of a record of time intervals between signals caused by irregularly falling drops. The signals are indicated below the interval record. The same interval pattern is recorded twice, to the left on film moving at high speed, to the right at low speed. The signal from the first drop rapidly carries the spot of the mirror galvanometer to the base of the record, writing a thin vertical line on the film. The spot then ascends more slowly producing a steeply rising line which is broader than the vertical line. The length of each steeply rising line is proportional to the time-interval between two drops. Furthermore, all such lines make the same angle with the base line. The interval record, therefore, has two time scales: one is expanded along the ordinate, the other consequently condensed along the abscissa. This system permits the recording of rapid effects on slowly moving film. As is illustrated on the right the thin vertical lines are usually not seen. By joining the upper ends of the lines, one obtains a curve which is inversely proportional to blood flow.

During the flow measurements the blood from the thyroid cannula was re-injected by means of drop-infusion into the femoral vein, either at a rate approximately equal to and varying with the outflow or at a constant rate over periods of measurements. The blood taken for radioiodine analysis was replaced by either dextran ('Macrodex'), 0.9 per cent sodium chloride or blood from another animal. In the latter case, the donor animal was anaesthetized in the same way as the recipient, heparinized and injected intravenously with large doses of glucose solution, in an attempt to reduce the adrenaline response to bleeding. If large amounts of blood were needed the donor was given dextran or glucose before the blood pressure had fallen to critical levels.

In cases where intravenous fluid was given, glucose was preferred to sodium chloride, because the latter easily caused electrolytic imbalance.

The collection of blood samples for the assay of radioactive substance

Each drop of blood from the free end of the cannula inserted in the thyroid vein fell on a slowly running strip of filter paper (LKB 'testrulle,' TR 3391), which was advanced continuously and kept perfectly flat by a device similar to the film transport mechanism of a camera. The blood drops were then dried by a stream of hot air before the paper was wound on the 'exposed' roll. Drops of blood that had passed through the thyroid could thereby be preserved for detailed analysis afterwards in the order which they had appeared at the end of the cannula. The measurement of the radioactivity of the blood drops was also found to give more accurate values than those obtained with a Geiger-Müller tube recording the radioactive content of the thyroid cannula directly. The time of measurement could thereby be adjusted to the activity of the drops and was not dependent on the rate of flow. Since the blood drops could be tested repeatedly, several isotopes with different half-lives could also be used simultaneously and recognized afterwards from the rate of decay of the radioactivity in each drop. This method was also used for the analysis of urine samples.

The arterial concentration of radioactive isotopes was determined with a technique similar to that used for the measurement of the thyroid venous blood. The drops were obtained from a thin cannula inserted into one of the iliac arteries. A high resistance in the cannula reduced the rate of flow. Venous blood from indifferent areas was also sometimes tested as an index of the arterial concentration. This could be done when blood concentration of the administered isotope had reached equilibrium with the tissue (RIGGS, 1952). The relation between the size of the arterial and the venous blood drops was determined repeatedly from measurements of known volumes of blood taken with pipettes, placed on filter paper and analyzed together with the blood drops.

The thyroid cannula and the thyroid veins between the gland and the cannula had a volume of between 3 and 10 drops, the largest space generally being in the cannula. Consequently, the analysis of a drop which appears at the end of the cannula does not show what is happening in the thyroid at that very moment but at an

Fig. 3. Flow profile of contrast material in a vessel during flow in the direction of the arrow, showing the optically diffuse border. (From BRECHER, 1956, with permission of Grune and Stratton, Inc., New York.)

earlier time when that particular amount of blood was passing through the capillaries of the gland. If blood moved through the tubing like a solid rod one would only have to make a correction for the exact volume of the cannula and the blood vessels between it and the active regions of the gland. However, flow in a thin tube is more or less irregular, with the slowest rate and sometimes with considerable turbulence near the walls. An example is presented in Fig. 3 where the profile of the interphase between two materials with different radiation density has been made visible. The contrast material that was suddenly administered flows like a paraboloid cone, illustrating that the axial stream was more rapid than that near the walls of the vessel. Similarly, changes in content of radioiodine in the thyroid blood drops generally appeared somewhat earlier than one would have expected from the calculation of the dead space of the recording system and the effects were thereby somewhat smoothed. This artefact could never be completely avoided since the ratio between the rate of the most rapid to the rate of the slowest stream apparently varied with the rate of the total flow in the cannula. An example of this effect is clearly seen in Fig. 56 where, according to the calculations, the effect of the administered acetylcholine precedes or is simultaneous with the intravenous injection of the substance.

The radioactivity in the blood drops was determined with a scintillation counter which had a thallium activated sodium iodide crystal 4 cm in diameter and 25 mm thick in front of an E. M. I. photomultiplier, type 6260. The gamma counter operated an EKCO scaler type N 530. In a few experiments a β-sensitive large end-window G–M tube (Amperex) was used instead of the gamma counter.

A cesium-137 standard (0.5 μC Sc^{137}; half-life 33 years) was used to calibrate the sensitivity of the detecting instrument. To get comparable accuracy of consecutive measurements, the time required for the instrument to register a certain number of counts was recorded. Besides the variations in counts recorded from repeated measurements of one drop in a fixed geometrical position in relation to the crystal, there was a relatively small error due to difficulties in placing the drop in the optimal position in front of the crystal. The error due to variations in size of the drops, which could exceed 10 per cent if the drop rate was low, was relatively greater than the errors of measurement described above. This error could not be eliminated since in the calculation of the rate of uptake or the rate of release, the A–V difference corresponding to a certain drop was multiplied by a drop rate calculated from the distance between drops which had fallen earlier on the filter paper, as a correction had to be made for the volume of the recording system (see p. 12). The error was also considerably enlarged by the calculation procedure which contained subtractions and multiplications.

Radioactive isotopes of iodine

Two isotopes of iodine were used in these experiments. The first was I^{131} (obtained from the Radiochemical Centre, Amersham, England) which has a half-life of 8 days. The other, used in a few experiments, was I^{132}, which has a half-life of 2.3 hours. The I^{132} was obtained from Dr. L.-G. Larsson and Dr. J. Einhorn, Radiumhemmet, Karolinska Sjukhuset, Stockholm, who recovered the isotope as a decay product of Te^{132}. They separated the iodine from tellurium by simple distillation, using a modification of the technique described by COOK, EAKINS and VEALL (1956). The two isotopes of iodine, I^{131} and I^{132}, could be distinguished in the blood drops by measuring the radioactive decay of each sample of blood. As Te^{132} is always somewhat contaminated with Te^{131} the I^{132} is never quite pure. Since the ratio I^{132}/I^{131} is steadily decreasing in a mixture of the two, the first measurement of the blood samples had to be done as soon as possible during the experiment to reduce the error to a minimum.

Brain stimulation

Local heating of the hypothalamus was produced by passing a high frequency alternating current (1 megacycle per sec) of low voltage between enamelled steel needles insulated to within two mm of their tips. The electrodes were mounted together in pairs with an inter-needle distance in each pair of two mm and a distance of four mm between the pairs. A coil of low ohmic resistance (55 ohms) shunted the pairs of electrodes to reduce the effect of any rectification in the tissue. Four needles were mounted together on the manipulator of a stereotaxic instrument. At the bare tip of one of the needles a thin enamelled constantan wire (0.1 mm in diameter) was soldered to form one end of a thermocouple by which the maximal temperature produced by the heating current could be recorded. The reference point was placed in melting ice. The thermocurrent was compensated with a fraction of the current from a galvanic cell so as to set the galvanometer zero point at about 37°C. The equipment has previously been used by C. VON EULER and SÖDERBERG (1957). Similar techniques were described by MAGOUN, HARRISON, BROBECK and RANSON (1938), FOLKOW, STRÖM and UVNÄS (1949) and C. VON EULER (1950).

Cutaneous and rectal temperatures were also recorded thermoelectrically with mirror galvanometers.

Electrodes for brain stem stimulation were orientated with a Horsley-Clarke stereotaxic instrument in the experiments on cats. In the rabbits a modified technique was used by which the electrodes were inserted in relation to the plane of the tangents through the bregma and kept in position with metacrylate dental cement ('Swedon'). The acrylate was also used for implantation of cortical silver ball electrodes for the electroencephalogram (EEG) which was recorded by a six channel electroencephalograph (Offner, type A).

Histological control

The lateral lobes from 15 rabbits were removed at the end of the experiments, fixed in 10 per cent formalin, embedded in paraffin, sectioned and then stained with haematoxylin-eosin or with 'azan' according to HEIDENHAIN (1915).

RESULTS

Thyroid blood flow

The rate of thyroid blood flow under resting conditions was found to be more or less constant for considerable periods of time. The flow varied markedly from animal to animal and seemed to be faster in the autumn and winter than in the summer. In Fig. 4 the resting level of flow in 55 rabbits measured during 1957 is graphically compared to the time of the year in which the experiments were performed. Below the abscissa the average rate of flow for each month is given. No measurements of this kind were made in July and August but other rabbit experiments during that period revealed glands that were very small and had narrow veins suggestive of low rates of flow. Furthermore, glandular weight and rate of flow, as judged by inspection, increased rapidly if the animals were kept at a room temperature of + 4°C.

Thyroid blood flow was also considerably higher in rabbits than in cats, both in relation to body weight and to the weight of the perfused glands. This difference was not due to difference in oxygen consumption of the glands in the two species since the venous blood from the rabbit thyroid was only slightly darker than the arterial blood whereas the thyroid venous blood of the cat was generally as dark as the blood in the *vena cava.*

Vasomotor reactions in response to various stimuli described below were also often more marked in the rabbits than in the cats. However, no real qualitative difference could be demonstrated since the degree of vasomotor response to a certain stimulus seemed to be closely related to the resting level of the flow and this was much lower in

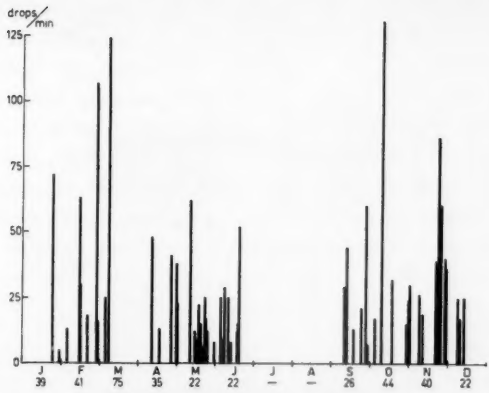

Fig. 4. The resting level of blood flow from one lobe of the thyroid in 55 rabbits measured during 1957. *Ordinate:* Blood flow in drops per min. *Abscissa:* Time of the year when the experiments were performed. Below each month the average rate of flow is given. No measurements in July or August. The animals were randomly chosen with the exception that those whose thyroid veins were too small to cannulate were rejected.

the cats. Thus, summer rabbits and those in which the thyroid activity had been reduced artificially showed reactions similar to those obtained in normal cats.

Sympathetic nerves

Electrical stimulation of the cervical portion of the ipsilateral sympathetic trunk invariably elicited vasoconstriction in the thyroid. The effect generally outlasted the period of stimulation, sometimes for several minutes as can be seen in Fig. 5 from a rabbit under light Nembutal. The cranial portion of the cervical sympathetic was cut in this experiment to avoid the fall in systemic blood pressure which appears when sympathetic fibres to the baroceptors in the carotid sinus are stimulated (PALME, 1943; LANDGREN, 1952). The effect of 2 μg adrenaline in the same animal is shown in *A* for comparison. Only very high single doses of adrenaline or noradrenaline

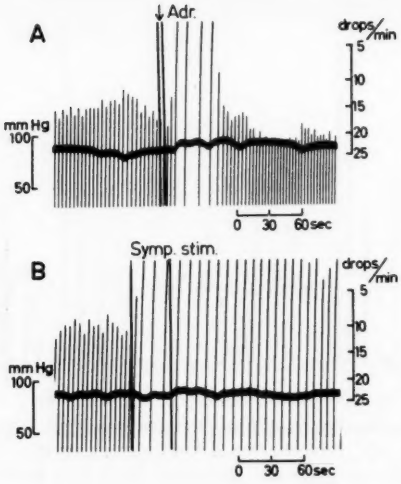

Fig. 5. Rabbit 4.7 kg. Nembutal. Blood flow from left thyroid lobe (calibration to the right in drops per min). Blood pressure of femoral artery (calibration to the left). *A*, 2 μg adrenaline i. v. at arrow. *B*, between thick vertical lines, electrical stimulation of the cranial portion of the cut left cervical sympathetic trunk with 2 volts, 10 per sec.

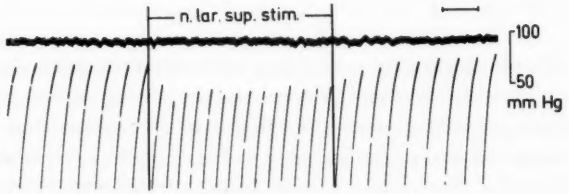

Fig. 6. Cat. 3.3 kg. Nembutal. Femoral blood pressure (calibration to the right) and blood flow from the left thyroid lobe (no calibration) Between thick vertical lines, electrical stimulation of the peripheral end of the cut left superior laryngeal nerve (2 volts, 30 per sec). Horizontal bar in the upper right corner = 1 min.

could elicit vasoconstrictions comparable in duration and magnitude to those of stimulation of the sympathetic nerve (*cf.* below).

The effect of sympathetic stimulation, in good agreement with the anatomical situation described in "Methods," was sometimes partly abolished by cutting the laryngeal nerves. Only minute effects could be induced by stimulation of the contralateral sympathetic. It should however be remembered that the experiments represent either the flow from thyroids of animals with very marked symmetry or from the more developed side of asymmetrical thyroids.

Superior laryngeal nerve

Stimulation of the peripheral end of the cut superior laryngeal nerve often evoked vasodilatation of thyroid vessels. In this case it rarely outlasted the period of stimulation for as long as after stimulation of the sympathetic but was well maintained at a constant level during the stimulation period. This is illustrated in Fig. 6 from a cat under Nembutal. The effect of stimulation of the laryngeal nerve was sometimes mixed (see *e. g.* Fig. 50 on p. 64) and sometimes a slight vasoconstriction was the only result seen, confirming the old observation that in this nerve there are also sympathetic fibres. It is possible that selective stimulation either by thermal stimuli (C. VON EULER, 1947) or by using anodal block (*cf.* WIDÉN, 1955) might have served to elucidate the action of different types of fibres in thyroid nerves. Such experiments have not yet been done but some information on this point has been obtained by studying reflex activities (see below).

The recurrent nerve

Only in one out of ten animals did stimulation of the recurrent nerve produce vasomotor changes. The effect is demonstrated in Fig. 7 which shows a marked vasodilatation. This rabbit, anaesthetized with Nembutal-chloralose, was in poor condition and repeated tests could not be carried out. It has previously been assumed that there are both dilatator and constrictor fibres in the recurrent nerve, but changes in parameters of stimulation never revealed anything of this kind nor was vasoconstriction ever observed.

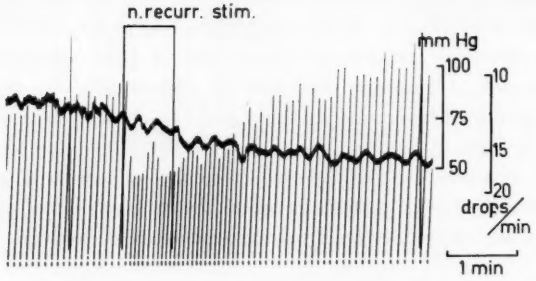

Fig. 7. Rabbit. 2.7 kg. Nembutal-chloralose. Femoral blood pressure and blood flow from the left thyroid lobe (calibrations to the right). At the mark, electrical stimulation of the peripheral end of the cut ipsilateral recurrent nerve (2 volts, 50 per sec).

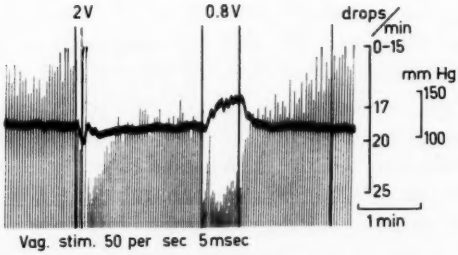

Fig. 8. Rabbit. 3.0 kg. Nembutal. Femoral blood pressure and blood flow from the left thyroid lobe. Between heavy vertical lines, electrical stimulation of the cranial end of the cut vagus nerve on the left side. Stimulus strength indicated in the figure.

Reflexes from the vagus

Electrical stimulation of the cranial end of the vagus, cut below the superior laryngeal nerve, generally had a very marked influence on thyroid blood flow. Two examples will be discussed. Part of one is seen in Fig. 8 from a rabbit under Nembutal. Vagal stimulation at frequencies from 50 to 200 per sec elicited thyroid vasodilatation with a latency shorter than the interval between two drops (3 to 5

sec). The figure shows two such vasodilatations. The first was obtained from a strong stimulus which was probably nociceptive and 'aroused' the animal. It was followed by a slight immediate thyroid vasodilatation poorly seen in the figure, and after a latency of 10 sec or more, by a marked dilatation. The thyroid vasomotor tone was still somewhat reduced at the onset of the second stimulation two min after the first one. The second was weaker and did not provoke 'arousal' but caused a marked retardation of the respiratory frequency, an immediate increase of thyroid blood flow and a gradual rise of the systemic blood pressure. Low frequency stimulation, which had no effect on the thyroid blood flow or merely provoked a slight reduction of it, markedly accelerated the rate of respiration.

The other example (Fig. 9) is taken from an experiment on an *encéphale isolé* cat with stimulating electrodes on the ipsilateral vagus below the superior laryngeal nerve (the vagus cut below the electrodes). It shows an effect similar to that of Fig. 8 but also demonstrates that the vasodilatation is mediated by the superior laryngeal nerve which is intact in *A* where the vagal stimulation is followed by reduced thyroid vasomotor tone, but cut in *B* where the thyroid vasomotor response is abolished.

Both arterial pressure and blood volume influence thyroid vasomotor tone. The experiments of REIN, LIEBERMEISTER and SCHNEIDER (1932) have shown that thyroid vasodilatation could be reflexely elicited from the carotid sinus. In the present study it was frequently observed that the blood flow in the innervated thyroid was far more sensitive to changes in arterial pressure than the blood flow in the denervated thyroid and in other regions of the body. On the other hand, considerable changes in thyroid blood flow could also be elicited by intravenous infusions which did not affect the systemic blood pressure or which even lowered it. This is shown in Fig. 10 from a rabbit of 4.2 kg. Ten ml isotonic glucose solution injected intravenously caused a prompt increase in blood flow and only a slight rise in blood pressure. Below the flow record the drops of urine from the left ureter are indicated (u). It was evident from the original record (over a longer period of time than is reproduced in the figure) that the intervals between urine drops after glucose administration were shorter than those before. (See also Fig. 49 on p. 63 illustrating the

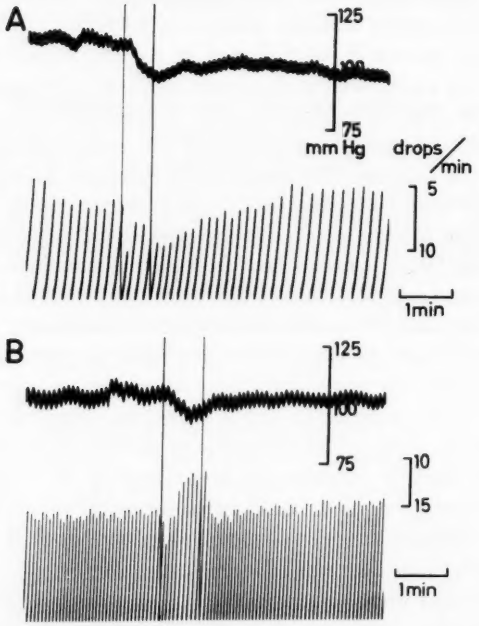

Fig. 9. Cat. 2.2 kg. *Encéphale isolé.* Blood pressure and flow from left thyroid lobe. *A*, before, *B*, after section of the superior laryngeal nerve. Between marks, electrical stimulation of the cranial end of the cut ipsilateral vagus (4 volts, 50 per sec).

accelerated rate of thyroid flow from 20 to 70 drops per min provoked by an increased rate of the intravenous re-infusion to two or three times that of the venous outflow.) The rate of intravenous re-infusion that compensated for the blood loss through the thyroid cannula had thus to be carefully adjusted. The high sensitivity of the thyroid vessels to changes in arterial pressure and blood volume made pharmacological studies of the gland very difficult.

Oscillatory behaviour was sometimes observed in the thyroid flow. It may have been caused by baroreceptor reflexes. Oscillation is in fact a frequently occurring phenomenon in the blood pressure homeostasis observed as early as 1865 by LUDWIG TRAUBE. It is not un-

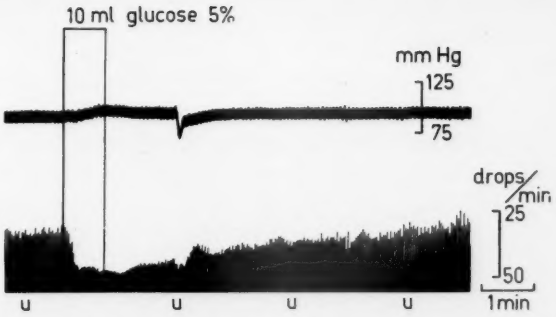

Fig. 10. Rabbit. 4.2 kg. Nembutal-chloralose. Blood pressure in femoral artery and blood flow from right thyroid lobe. Under flow record the letter "u" signifies the moment when each drop of urine fell from a catheter in the luft ureter. The injection of 10 ml 5% glucose solution slightly increased systemic blood pressure and doubled thyroid blood flow. Transient increase in urine flow appeared simultaneously (*cf.* text).

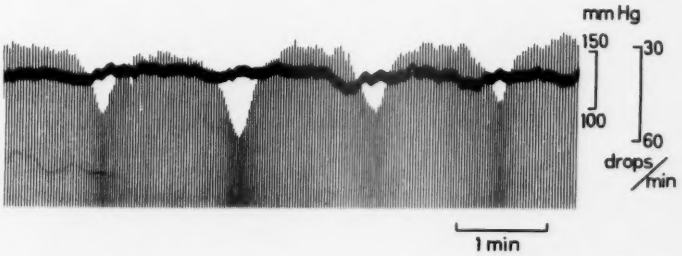

Fig. 11. Cat. 5.4 kg. Nembutal. Femoral blood pressure and blood flow from left thyroid lobe. Note regular oscillations in pressure and flow.

expected therefore that in the thyroid, oscillations also appear which seem to reflect with high magnification the efferent activity induced by baroreceptors. The phenomenon is illustrated in Fig. 11 which shows a regular alternation in the rate of blood flow with a period of 80 sec which appeared 'spontaneously.' Similar fluctuations can be traced in the blood pressure record, the two processes, however,

being slightly out of phase in that the time of the lowest blood pressure preceded the moment of lowest thyroid vasomotor tone by 20 sec.

The oscillations often started when a sudden change in general body vasomotor tone was elicited *e. g.* by drugs or hormones. An example is reproduced in Fig. 19 where the effect was caused by acetylcholine and could be repeated several times with the same stimulus (see p. 30).

'The arousal reaction'

In the text, no distinction is made between an 'arousal' observed as change in the animal's behaviour, or one which was manifest only in the electroencephalogram. Most were of the first type.

In the rabbit 'arousal reactions' appearing spontaneously or elicited by twisting the pinna or by *weak* nociceptive stimuli were commonly accompanied by thyroid vasodilatation. It was found that the dilatation was mediated both by the sympathetic and by the superior laryngeal nerve from the vagus.

Fig. 12 is taken from an experiment on a rabbit in which all nerves were cut except the sympathetic fibres accompanying blood vessels. The irregularities seen in the drop interval record are artefacts caused by movements of the animal. The 'arousal' appeared 'spontaneously.' Note especially the period of slightly increased vasomotor tone which preceded the 'arousal' by about half a minute. In fact, such periods of increased vasomotor tone were often the first manifestations of shallow anaesthesia. *Strong* nociceptive stimuli in the rabbit were regularly accompanied by vasoconstriction. Electrical stimulation of the brain stem elicited a similar response. This is shown in Fig. 13 from a rabbit with stimulating electrodes in the mesencephalic reticular formation. The long-lasting vasoconstriction with only a transient rise of the systemic blood pressure could easily be reproduced by cervical sympathetic stimulation (*cf.* Fig. 5), but not with physiological doses of adrenaline (see p. 28). A similar response could also be obtained with antidiuretic hormone (*cf.* below) but only in doses of 50 mU per kg or more.

Nociceptive stimulation and electrical stimulation of the reticular formation of the mid-brain of the cat (the latter illustrated in Fig.

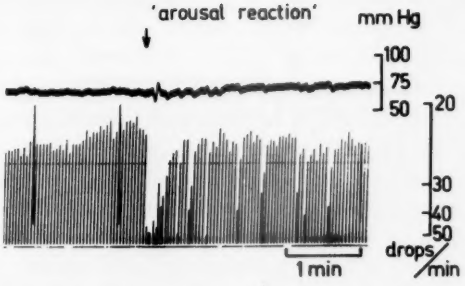

Fig. 12. Rabbit. 3.2 kg. Nembutal. Femoral blood pressure and flow from left thyroid lobe. The lobe was denervated except for sympathetic fibres accompanying blood vessels. At the arrow an 'arousal reaction' began, as judged from the behaviour of the animal.

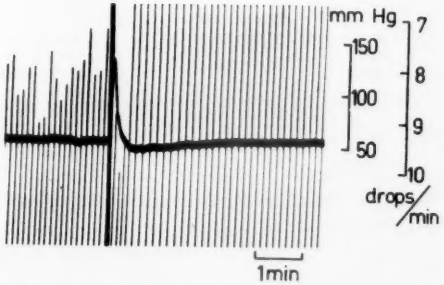

Fig. 13. Rabbit 2.0 kg. Nembutal-chloralose. Stimulating electrodes in hypothalamus and in the mesencephalic reticular formation. Femoral blood pressure and blood flow from left thyroid lobe. At the mark the reticular formation was stimulated with 2 volts, 100 per sec for a period of 5 sec. Note rapid transient increase in blood pressure and prolonged reduction of thyroid blood flow.

14) had less effect on thyroid blood flow than in the rabbit. The response was, however, very similar to that of adrenaline in cats. Note the late vasodilatation which also occurs after adrenaline. Sympathetic stimulation and antidiuretic hormone evoked similar marked vasoconstriction in the cat.

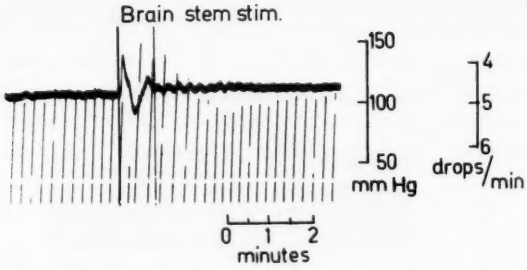

Fig. 14. Cat. 3.3 kg. Nembutal. Femoral blood pressure and blood flow from left thyroid lobe. Between marks electrical stimulation of the mesencephalic reticular formation (7 volts, 200 per sec).

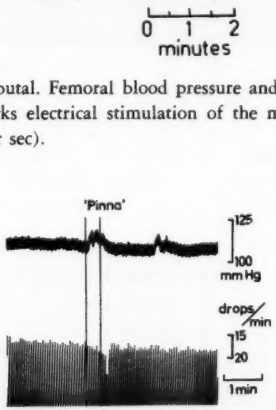

Fig. 15. Cat. 2.2 kg. *Encéphale isolé* prepared under Pentothal. Vagus and cranial sympathetic truncs cut bilaterally. Femoral blood pressure and blood flow from left thyroid lobe. Between marks, pinna was pinched.

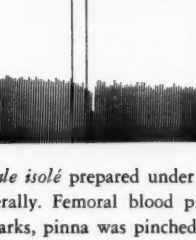

Fig. 16. Cat. 4.5 kg. *Encéphale isolé* prepared under Pentothal. Thyroid innervation intact except for sympathetic pathways which are interrupted by spinal cord section Femoral blood pressure and blood flow from left thyroid lobe. Between second and third vertical marks, twisting the pinna which caused signs of behavioural 'arousal' was followed by slight transient thyroid vasodilatation.

Hormonal events accompanying or causing 'arousal reactions' have often been reported in the literature (INGVAR, 1955; PURPURA, 1956; INGVAR and SÖDERBERG, 1958). Thyroid blood flow has not been found to be a sensitive indicator of such events. The only good example of such a reaction is reproduced in Fig. 15 from an *encéphale isolé* cat with the vagal nerves sectioned bilaterally. Pinching (not twisting) the pinna elicited sudden transient increases in systemic blood pressure, probably with an elevation of thyroid vasomotor tone although the latter is not statistically significant. Judging by the size of the blood pressure response the most likely explanation of the effect is that it was induced by liberation of catechols in the head. For comparison the effect of twisting the pinna of an *encéphale isolé* cat with intact thyroid innervation, in which the usual 'arousal' is accompanied by vasodilatation, is shown in Fig. 16.

Hypothalamic heating

In a few cases local heating of the anterior part of the hypothalamus elicited vasoconstriction in the thyroid. This is illustrated in Fig. 17 from a cat under Nembutal. The thyroid gland of the animal was small and the rate of flow from the left lobe of the gland was as low as 2 drops per min. When the hypothalamus was warmed from 37° to 43°C the time intervals between the drops increased for about three min and the blood flow fell to one drop per min. Two min after the onset of this thyroid vasoconstriction the temperature of the skin of the ear increased from 28° to 34°C indicating maximal vasodilatation (room temperature was 23°C). A slight fall in systemic blood pressure accompanied the effect. After cessation of heating the cutaneous temperature fell and the thyroid blood flow gradually returned to the initial level. This is in good agreement with observations by DIETRICH and SCHWIEGK (1932) who measured the flow with REIN'S "Thermostromuhr." They found that cooling the animal itself or the blood of the common carotid artery (dog under chloralose) increased thyroid blood flow and that heating caused a reduction. The effect was very sensitive to anaesthesia.

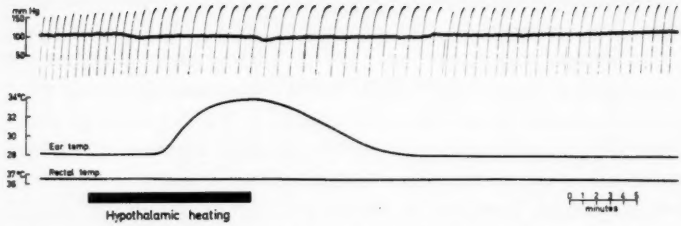

Fig. 17. Cat. 3.3 kg. Nembutal *Records from above downwards:* Thyroid blood flow from left thyroid lobe, femoral blood pressure, ear skin temperature and rectal temperature. Black bar below records indicates period of hypothalamic heating with high frequency alternating current, from 37°C to 43°C. Note reduced drop frequency (thyroid vasoconstriction) concomitant with period of increased ear skin temperature which indicates cutaneous vasodilatation.

The thyroid vasomotor response to hypothalamic heating appeared but occasionally although cutaneous vasodilatation was a regular event. It has not been possible to exclude that the thyroid vasoconstriction after hypothalamic heating might be caused by activity of baroreceptors or 'blood volume receptors.' Stimulation of posterior pituitary cells delivering antidiuretic hormone should also be considered.

Heating the whole animal sometimes also elicited thyroid vasoconstriction. However, the correlation with cutaneous vasomotor activity or shivering was never particularly good.

Adrenaline and noradrenaline

Intravenous or intra-arterial administration of l-adrenaline or l-noradrenaline generally induced thyroid vasoconstriction and often a subsequent vasodilatation. The degree of vasoconstriction was proportional to the dose of the catechol injected but varied with the initial circulatory state within the thyroid. Thus, most pronounced vasoconstriction was elicited in animals with a high resting rate of blood flow, as can be seen in Fig. 18 where 2 μg adrenaline intravenously reduced the blood flow from 130 drops per min to 9 drops. After the constriction, the flow increased to 220 drops per min or about 7 ml

per min. If the latter figure represents half the gland then at least 10 ml of blood which is about $^1/_{15}$ of the total blood volume may pass through the entire thyroid in one minute. (The blood volume of normal rabbits has been estimated to be about 60 ml per kg by ARMIN, GRANT, PELS and REEVE (1952) and of anaesthetized rabbits to be 50 ml per kg by MCKINNA and MISIEWICS (1954).) This was the highest rate of flow observed in the present experiments. A similar potent affect of adrenaline has previously been found by OSSOKIN (1914) in the goiterous dogs frequently found in Bern, Switzerland. In a typical summer rabbit with a flow as low as a few drops per min there was hardly any constriction, even after 10 micrograms of adrenaline, and the subsequent dilatation dominated the picture.

As a thyroid vasoconstrictor, noradrenaline was five to ten times weaker than adrenaline. Vasoconstriction elicited by either of these hormones generally lasted about one min. When adrenaline was slowly infused, the provoked vasoconstriction showed progressive exhaustion. A good example of this effect is illustrated in Fig. 55 on p. 71.

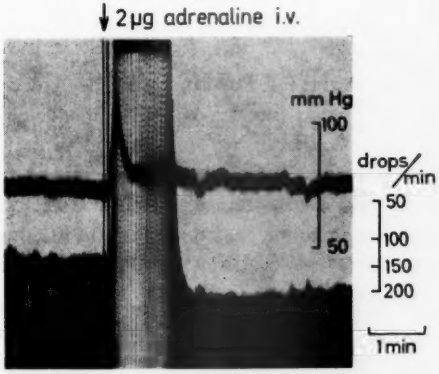

Fig. 18. Rabbit. 2.4 kg. Nembutal. Intact thyroid innervation. Femoral blood pressure and blood flow from left thyroid lobe. At arrow 2 µg adrenaline were injected intravenously.

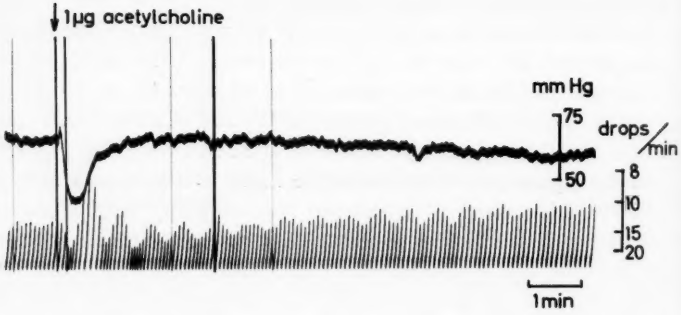

Fig. 19. Rabbit. 2.7 kg. Nembutal. Blood pressure in femoral artery and blood flow from left thyroid lobe. Intact thyroid innervation. At arrow 1 µg acetylcholine was injected intravenously.

Acetylcholine

The thyroid vascular response to acetylcholine in all animals was an immediate vasodilatation (see also Fig. 58 *C* on p. 74). If the innervation had been left intact, as was the case in the experiment of Fig. 19, a vasoconstrictor effect regularly followed dilatation. This has been interpreted as a sign of compensatory baroreceptor activity elicited by the general fall in vasomotor tone caused by the hormone. This effect on thyroid blood flow, when mediated by the nerves of the gland, often oscillated, in Fig. 19, with a period of 40 sec (*cf.* vagal reflexes on p. 22). As was demonstrated by ABRAHAMS and PICKFORD (1954) a vasoconstrictor effect with long latency would also occur if acetylcholine caused a release of antidiuretic hormone in large quantities (*cf.* also DUNCAN (1954) on release of adrenaline).

Serotonin (5-hydroxytryptamine)

Intravenously administered serotonin had no clear-cut effect on the thyroid. This may be due to the particular affinity of the lungs for this hormone (REID and RAND, 1951). Injected into the common

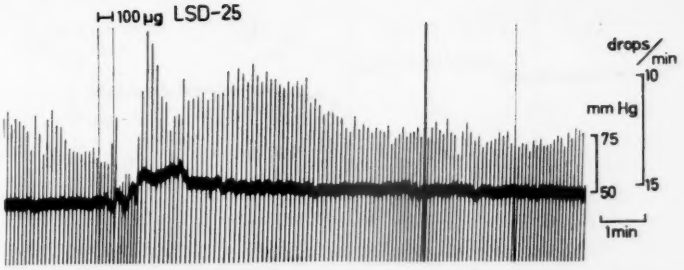

Fig. 20. Rabbit. 2.7 kg. Nembutal. Blood flow from left thyroid lobe and femoral blood pressure. Between marks, 100 µg lysergic acid diethylamide were injected intravenously.

carotid artery, however, serotonin dilated the thyroid vessels, but the effect was 10 times weaker than that of acetylcholine (*cf.* Fig. 58 on p. 74). Derivatives of lysergic acid which are known to be potent serotonin inhibitors (for references ROTHLIN, 1957; TROXLER and HOFMANN, 1957) showed, however, in some preliminary experiments, a variety of effects that could not be related to inhibition of any physiologically occurring hormone. Lysergic acid diethylamide induced a transient vasoconstriction which appeared with a latency as long as 30 sec or more in the rabbits tested (Fig. 20). Brom-lysergic acid diethylamide, on the other hand, which is a more potent serotonin antagonist than LSD, had a marked vasodilating effect (see Fig. 52 on p. 66). These effects have as yet been found only in rabbits. The only cat thyroid on which they were studied was insensitive to the drug.

Reflex discharge of catechols from the adrenal medulla

Decreased oxygen tension in the blood is known to increase the catechol output of the adrenals (RAPELA and HOUSSAY, 1952; U. S. VON EULER and FOLKOW, 1953). Thus asphyxia may cause a release of catechols of more than 2 micrograms per min per kg body weight in the cat. In the present experiments inhalation of a gas mixture of 8 per cent oxygen in nitrogen evoked an increase in systemic

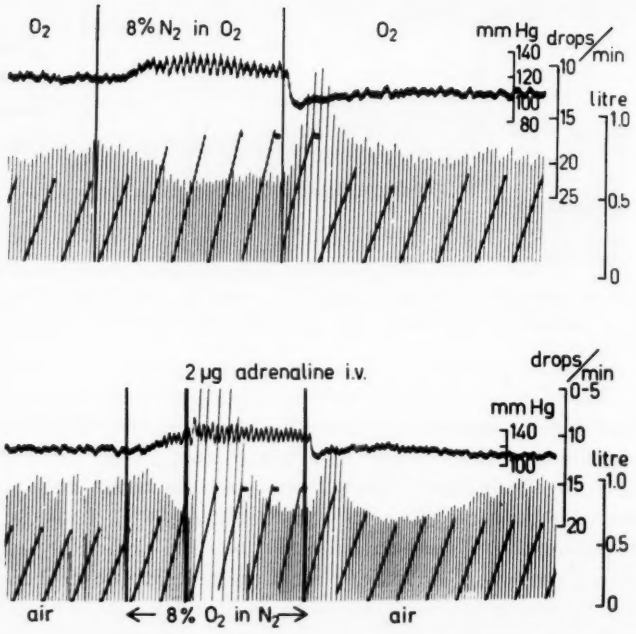

Fig. 21. Rabbit. 3.0 kg. Nembutal-chloralose. Femoral blood pressure and blood flow from left thyroid lobe. Thick oblique lines projected on the flow record reproduce the step-wise increasing volume of a spirometer that is emptied every 30 sec (calibration to the right in litres). *Upper graph:* The effect of a period of oxygen lack compared with the preceding and following periods of breathing pure oxygen. *Lower graph:* Periods of breathing air, 8 % oxygen in nitrogen, and air respectively, are indicated below the record. At second thick vertical line 2 µg adrenaline was administered intravenously. The uptake of iodine during this period is graphically reproduced in Fig. 38 (filled circles).

blood pressure that was accompanied by only a slight rise of the thyroid vasomotor tone (Fig. 21), even in rabbits in which fractions of a microgram of adrenaline caused a measurable vasoconstriction. Rebreathing into a small rubber bulb for half a minute, as in the experiment of Fig. 22, led to slight vasoconstriction apparent 20 sec after cessation of rebreathing.

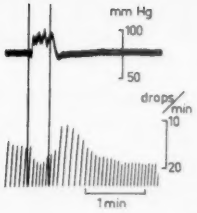

Fig. 22. Rabbit. 2.7 kg. Nembutal-chloralose. Femoral blood pressure and blood flow from left thyroid lobe. Between marks, rebreathing into a small rubber bulb.

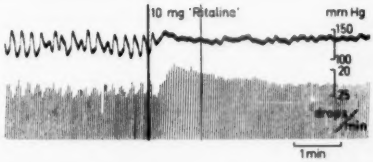

Fig. 23. Cat. 5.4 kg. Nembutal. Femoral blood pressure and blood flow from left thyroid lobe. At thick vertical line, intravenous injection of 10 mg ritalin ('Ritalina,' Ciba).

Increased catechol secretion has also been assumed to occur after administration of the stimulant ritalin ('Ritalina,' Ciba) (methyl-1-phenyl-2-piperidyl acetate). Ten mg of this substance injected intravenously in a cat (Fig. 23) also provoked an effect on blood pressure and thyroid blood flow that was identical with the effect of 10 micrograms of adrenaline given to the same animal.

Thyrotropic hormone (TSH)

In most experiments a Swedish commercial preparation of TSH ('Actyron,' Ferring) has been used. It could readily be administered intravenously without any undesirable effects on blood pressure or respiration. 'Thytropar' (Armour Laboratories, London) has also been tested in a few experiments. No systematic comparison was made.

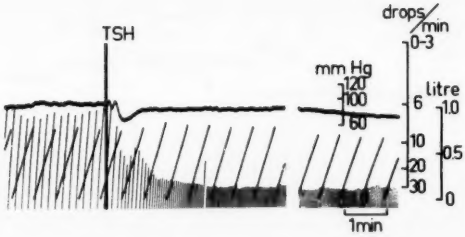

Fig. 24. Rabbit. 3.0 kg. Nembutal-chloralose. Femoral blood pressure and blood flow from left thyroid lobe. Oblique lines show the step-wise increasing volume of a spirometer that is emptied every 30 sec and hence graphically illustrate the tidal volume (*cf.* Fig. 21). The record is interrupted by a 5 min pause in the recording. At thick vertical line 4 U. S. P. units of TSH were injected into the femoral vein.

Two effects of the thyrotropic hormone have been observed. One was an immediate reduction of the vasomotor tone, sometimes of considerable magnitude in the rabbit. The systemic blood pressure generally responded with only a slight fall. This effect of reducing thyroid vasomotor tone was more marked late in the experiments than at the beginning when the titre of endogenous TSH would be higher. Such a rapid effect of TSH on the thyroid does not, however, parallel any other changes in activity after TSH and effects of impurities in the preparation cannot be excluded. Fig. 24 demonstrates the action of 4 U. S. P. units of TSH ('Actyron,' Ferring) on a rabbit 130 min after the beginning of the operation. A record of the respiration is also reproduced to show that the rate of breathing was unaffected, in sharp contrast to the case after injection of foreign proteins.

The other effect which also is still somewhat uncertain is a progressive reduction of the vascular resistance in the thyroid. It is easier to observe this slow effect in cats than in rabbits since the former have a more stable rate of flow than the latter. The cats which were not treated with TSH, in contrast to the treated animals, generally showed an increasing thyroid vasomotor tone. However, further experimentation is required to elucidate these reactions. Their interpretation is particularly difficult because of the great sensitivity of the thyroid to baroreceptive activity.

Antidiuretic hormone (ADH)

Intravenously administered pituitary antidiuretic hormone immediately induced a marked and prolonged vasoconstriction in the thyroid. The effect was not mediated by nerves or by adrenaline or noradrenaline from the suprarenals, nor was it influenced by large doses of dihydro-ergotamine. The constriction was never followed by 'reactive hyperemia.' The response varied considerably from animal to animal but not in parallel with the response to adrenaline, and it was as pronounced in cats as in rabbits. In some animals 50–250 mU ADH were more potent than 5 micrograms of adrenaline (Figs. 25–26); in others 2 000 mU had to be injected to obtain a response comparable to that of a few micrograms of adrenaline (Fig. 27). The injection of nicotine or hypertonic salt solutions which are known to release ADH from the pituitary (BURN, TRUELOVE and BURN, 1945; VERNEY, 1947) sometimes also provoked thyroid vasoconstriction but with a latency of 30 to 40 sec.

Oxytocin and adrenocorticotropic hormone (ACTH) produced a slight vasoconstriction which may probably be explained as due to contamination with ADH. The effect of 500 mU oxytocin is shown in Fig. 28.

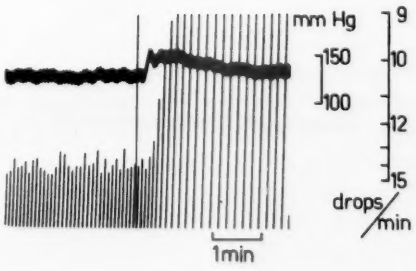

Fig. 25. Cat. 4.9 kg. Nembutal. Blood flow from right thyroid lobe. At vertical line, 250 mU antidiuretic hormone ('Pitressin,' Parke-Davis) were injected intravenously.

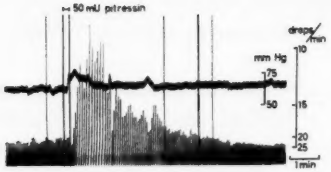

Fig. 26. Rabbit. 2.2 kg. Nembutal. Blood pressure from femoral artery and flow from left thyroid lobe. As indicated, 50 mU antidiuretic hormone injected intravenously.

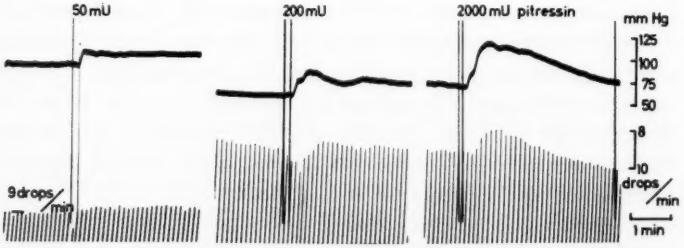

Fig. 27. Cat. 4.5 kg. *Encéphale isolé.* Femoral blood pressure and blood flow from left thyroid lobe. The effect of 50, 200 and 2 000 mU antidiuretic hormone injected intravenously as indicated. Time constant of drop interval recorder was changed between first and second record. Flow calibration of first record shown on the left, of the second and third records, on the right.

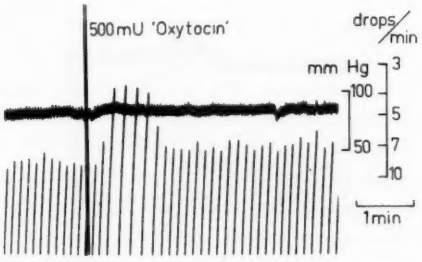

Fig. 28. Rabbit. 2.3 kg. Nembutal-chloralose. Femoral blood pressure and blood flow from left thyroid lobe. Between vertical lines, 500 mU oxytocin (Parke-Davis) were injected intravenously.

Adrenal cortical hormones

Cortisone, hydrocortisone and desoxycorticosterone acetate (DOCA) had no effect on thyroid blood vessels. However, the intravenous administration of 5 mg DOCA ('Percorten,' Ciba) rapidly reversed the development of pulmonary oedema and shock in rabbits showing increased respiratory rate and decreased oxygen saturation of arterial blood.

Adrenocorticotropic hormone (ACTH)

ACTH had irregular effects on thyroid circulation. Seven out of 11 rabbits showed vasodilatation, three responded with pure vasoconstriction and one exhibited no effect. In six of the rabbits responding with dilatation, vasoconstriction appeared 20 to 60 sec after the onset of the dilation. One cat showed no thyroid vasomotor response. The effects could not be related to the dose or to the initial state of the blood flow. No attempts have been made to determine whether one or both of the effects are caused by impurities of the preparation. In one experiment the hormone was administered attached to sodium carboxymethylcellulose which is reported to prolong its action. The thyroid response was a dilation of more than 20 min duration, *i. e.* a significantly prolonged effect. (*Cf.* ROBERTS, 1957, for references on ACTH in the blood.)

Anaesthetics

The rapid injection of a small dose of barbiturate (Nembutal, Pentothal or Thiogenal) elicited a transient reduction of the vasomotor tone, shown in Fig. 29 which illustrates the effect on the rabbit thyroid. Chloralose, on the other hand, provoked a slight vasoconstriction (Fig. 29). The thyroid vasomotor tone was less sensitive to anaesthetics than the thermoregulatory tone of the skin as shown in a cat, Fig. 30. In the brain (INGVAR and SÖDERBERG, 1956) barbiturates abolish vasomotor reactions by producing a moderate vasoconstriction which cannot then be further influenced by other agents. This has not been seen in the thyroid.

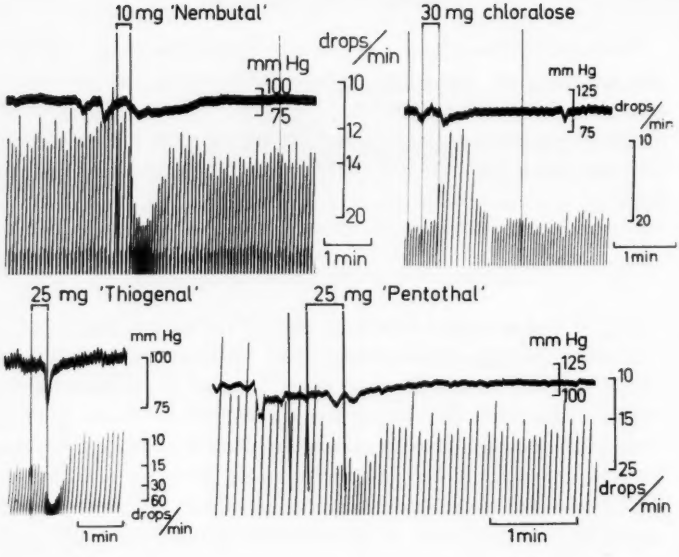

Fig. 29. The effect of four anaesthetics on femoral blood pressure and thyroid blood flow of rabbits under light Nembutal anaesthesia. (Body weight 2.2 to 2.7 kg.) Each record calibrated individually. Dose and name of anaesthetic agent indicated. ('Thiogenal' = sodium methylthioethyl-2-pentyl-thiobarbiturate, Merck, Darmstadt.)

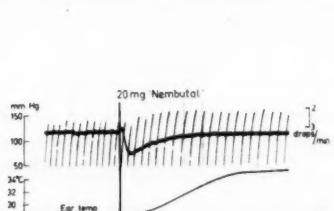

Fig. 30. Cat. 3.3 kg. Nembutal. *Records from above downwards:* Thyroid blood flow from the left lobe (calibration to the right), femoral blood pressure and ear skin temperature (calibrations to the left) and rectal temperature (calibration to the right). At thick vertical line, 20 mg Nembutal were injected intravenously.

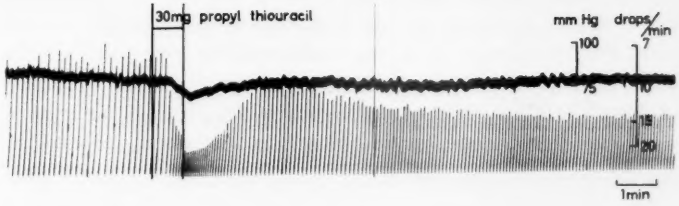

Fig. 31. Rabbit. 2.5 kg. Nembutal. Femoral blood pressure and blood flow from left thyroid lobe. Between marks, 30 mg propyl thiouracil were slowly injected intravenously.

Drugs inhibiting thyroid activity

Thiouracil was found to have very little effect on thyroid blood flow except for an acute but transient reduction in vasomotor tone when given in large doses intravenously, as in Fig. 31. However, part of the effect was due to some reflex activity in response to the large volume of fluid that had to be injected with the drug.

Far more interesting was the action of dimethylolmercaptobenzimidazol ('Matinol,' Byk-Gulden). Besides its antithyroid activity it is also reported to be a potent sedative (HÄUSLER, 1951). When this substance was given a few hours pre-operatively, enough anaesthesia was obtained with a very small dose of Nembutal to permit surgical incision. In a rabbit treated in this way thyroid blood flow was reduced to the lowest values seen in any experiment and on inspection, the gland was quite pale. The rabbit of Fig. 32 was given 400 mg Matinol subcutaneously at 14^h30. The anaesthesia became 'surgical' after 20 mg Nembutal had been injected intravenously at 16^h. An additional dose of 10 mg Nembutal was administered at 18^h45. The average rate of flow was between two and five drops per min from one half of the gland. A large dose of TSH was without effect. The record reproduced in Fig. 32 started at 19^h55 when altogether 80 mg Nembutal had been administered intravenously before the anaesthesia reached a lethal depth. The Nembutal considerably reduced the vasomotor tone as blood flow increased from

5 to 8 drops per min and blood pressure fell from 75 to 22 mm Hg. It has to be remembered that rabbits are very sensitive to Nembutal. Further injection of 30 mg would probably have killed a rabbit at the same level of barbiturate anaesthesia as that at the beginning of Fig. 32. When injected intravenously for the first time in an acute experiment under routine Nembutal anaesthesia, Matinol induced vasodilation (Fig. 33).

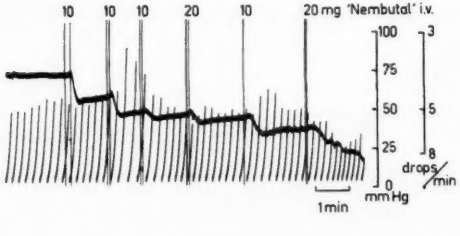

Fig. 32. Rabbit. 2.1 kg. 'Matinol'-Nembutal anaesthesia. Femoral blood pressure and thyroid blood flow. Six consecutive injections of Nembutal markedly reduced thyroid vasomotor tone (further discussion in the text).

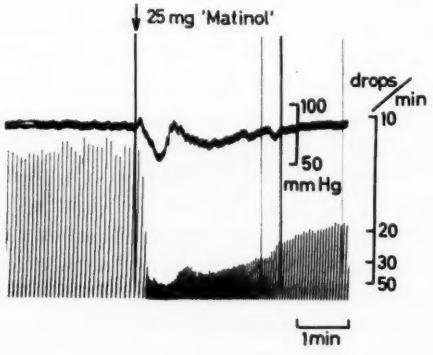

Fig. 33. Rabbit. 2.3 kg. Nembutal-chloralose. Femoral blood pressure and blood flow from left thyroid lobe. At arrow, 25 mg 'Matinol' were injected intravenously.

The uptake of inorganic iodine from the blood

In the experiments of BROWN-GRANT and GIBSON (1955) the iodine clearance of the rabbit thyroid gland, *i. e.* volume of blood cleared of iodine per unit time, was slightly below or of the same order of magnitude as the rate of blood flow found both in this and in earlier investigations. One would thus expect that the concentration of radioactive iodine in the thyroid vein would be much lower than in the arterial blood. In view of this, then, the accuracy of determinations of venous blood radioiodine was improved by the administration of relatively large doses of tracer, *i. e.* 50 to 200 μC I^{131}. As was expected, the venous radioiodine concentration was very often found to be less than half the arterial concentration; the arteriovenous (A–V) difference was highest immediately after the administration of the tracer, and then slowly declined. In one experiment the venous concentration, [V], was as low as 15 per cent of the arterial value, [A], and in several others between 15 and 20 per cent. As the clearance can be expressed by

$$\frac{[A]-[V]}{[A]} \times \text{Flow} \qquad (1)$$

it was, in the first case mentioned, 85 per cent of the flow value. Very little uptake of iodine occurs in extra-thyroidal tissues in the region studied. When several animals were compared, clearance was not proportional to flow but varied considerably, although the dissection was always carefully performed, and several controls to exclude extra-thyroidal anastomoses were made. The thyroid A–V difference of radioiodine was sometimes very small. This was more often the case in the summer than in the winter. Consequently, the first factor in the expression (1):

$$\frac{[A]-[V]}{[A]} \qquad (2)$$

sometimes called the extraction efficiency (POCHIN, 1950; RIGGS, 1952), is an index of thyroid function that is not always closely related to the clearance. POCHIN (1950) calculated thyroid flow

from measurements of the extraction efficiency in man, the values for the two being 6 ml per g of thyroid per min and 0.2 respectively in his patients.

As a control for the following experiments the arterial concentration of radioiodine was carefully measured with the drop technique for a long period of time while the test procedures were tried out. It was found that the arterial concentration did not change significantly within periods of 5 to 10 min provided that the rate of compensatory venous infusion was low and that distribution equilibrium for radioiodine was well enough established. Therefore, in those acute tests to be described which only last for periods less than a few minutes, venous concentration [V], A–V difference [A] – [V] or rate of uptake ([A] – [V]) × Flow, will be reproduced. The A–V difference is thereby proportional to the extraction efficiency and the rate of uptake is proportional to the clearance. In these two relationships the factor of proportionality is the arterial concentration, [A], which is constant for the limits set above.

Both the clearance and the extraction efficiency were highest immediately after the injection of the tracer. The latter decreased during the first 10 to 30 min after which the rate of decline was slow. However, both flow and clearance could be accelerated by TSH. As will be discussed on p. 91 the reduction of the clearance values is not necessarily an artefact due to output of labelled thyroid hormone. In the present experiments insignificant amounts of labelled hormone were released from most rabbit thyroids unless exogenous TSH was administered (see p. 60–61.).

The A–V difference of radioiodine

The arteriovenous concentration difference of radioiodine was followed continuously in 10 cats and 30 rabbits in which the thyroid nerves were stimulated and hormones or drugs administered. A typical example is shown in Fig. 34 which demonstrates the effect of 2.5 micrograms of adrenaline injected intravenously into a rabbit. *A* is a graph of the blood flow showing the usual vasoconstriction followed by a weak dilatation. The concentration of I^{131} in the thyroid vein *(B)* and its mirror image, the arteriovenous concentration

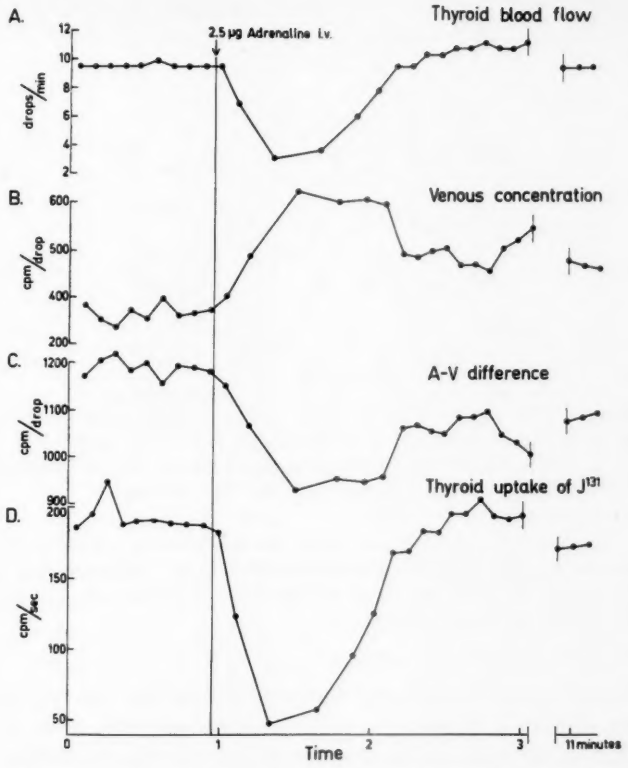

Fig. 34. Rabbit. 2.8 kg. Nembutal. Forty min before the record reproduced in the figure, 200 μC I^{131} were injected intravenously. *A* – *D* plotted on the same time scale with individual calibrations to the left. *A*, thyroid blood flow in drops per min calculated from the reciprocal value of drop intervals. *B*, venous I^{131} concentration in counts per min per drop. *C*, A–V diff. of I^{131} obtained by subtracting venous I^{131} from the average arterial concentration during the period. *D*, rate of thyroid uptake of I^{131} obtained by multiplying A–V difference by rate of blood flow. Abscissae in *B* and *C* were obtained by adding to the time each drop fell on the filter paper, a correction for time of passage through the outflow system. *A* and *D* have their points on the average time of the period they represent. This system will be used throughout the following figures. At arrow and vertical line, at time 1 min. 2.4 μg adrenaline, injected intravenously, caused thyroid vasoconstriction, reduction of A–V difference and of rate of uptake of I^{131}.

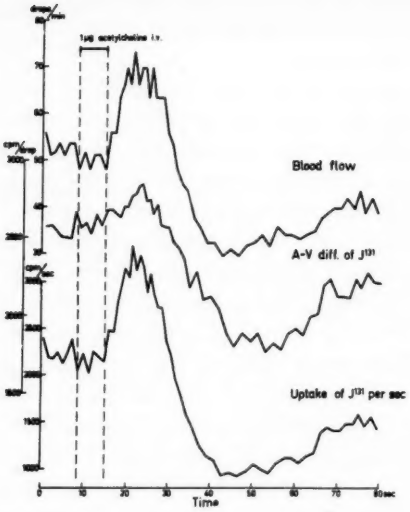

Fig. 35. Rabbit. 2.7 kg. Nembutal. Thirty min before record, 150 μC I^{131}. *From above downwards:* Thyroid blood flow from left lobe, A–V difference of I^{131} and rate of uptake of I^{131} (calibrations to the left). Between dashed lines, 1 μg acetylcholine intravenously. See also Fig. 36.

difference *(C)*, are indicated below in counts per min per drop (cpm/drop) *i. e.* radioactivity per unit volume of blood. If the A–V difference is multiplied by the rate of flow one obtains the rate of uptake of I^{131} shown in *D* in counts per min taken up by the thyroid every sec (cpm/sec). Clearly the A–V difference is reduced during the period of vasoconstriction. This was not a specific effect of adrenaline since it was not related to the dose of adrenaline administered but closely followed the rate of blood flow through the gland regardless of the stimulus that had elicited the change in circulation. Conversely, increased flow was accompanied by increased A–V difference. In fact, a simple rule could be traced indicating a slight correlation between flow and A–V difference as demonstrated in Fig. 35 and 36. Fig. 35 is a graphical reproduction similar to that in Fig. 34 but from an experiment in which 1 microgram of acetylcholine

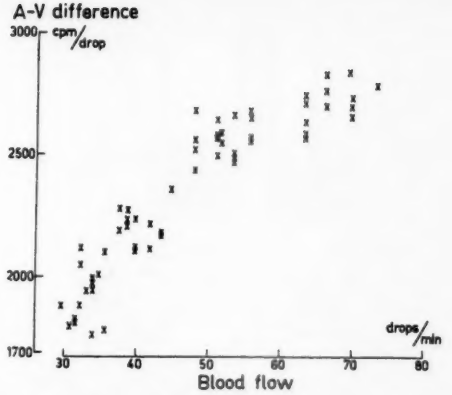

Fig. 36. The rabbit of Fig. 35. Graph of the relation between thyroid blood flow and A–V difference of I^{131} in the experiment of Fig. 35, when changes in flow were elicited both by vasomotor activity and by changes in the arterial blood pressure.

was injected intravenously into a rabbit. The uppermost curve shows first an initial increase of the rate of flow caused by dilatation and then a decrease secondary to the fall in the systemic blood pressure. The curves of A–V difference and the rate of uptake of iodine which are both plotted below the curve of the blood flow have largely the same shape as the latter. The relation between A–V difference and rate of flow is also graphically reproduced in Fig. 36 which demonstrates a curve approaching one asymptote parallel to the x-axis at high rates of flow and another parallel to the y-axis at low rates. The asymptotic values varied from animal to animal and were probably to a minor degree due to an artefact from blood of extra-thyroidal vascular beds. Supporting this statement is the observation that a reduction of flow caused by occluding the carotid artery did not always result in a reduced A–V difference but sometimes in an increase. This was the case in Fig. 37 where the original flow record and the venous concentration are reproduced. The latter fell from an average of 210 to 170 cpm/drop or about 20 per cent during the period of diminished arterial pressure in the

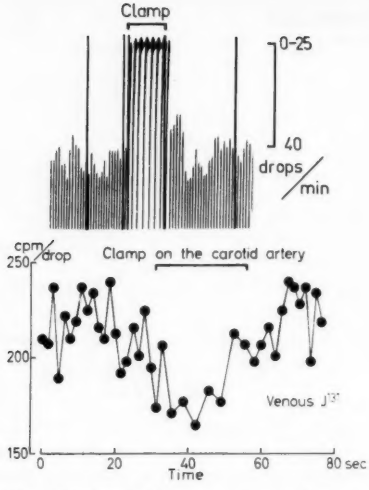

Fig. 37. Rabbit. 2.7 kg. Nembutal-chloralose. 15 min before record, 200 μC I^{131}. Original flow record from left thyroid lobe (above) and thyroid venous concentration of I^{131} (below). As indicated, the left common carotid artery was clamped caudal to the thyroidal branches.

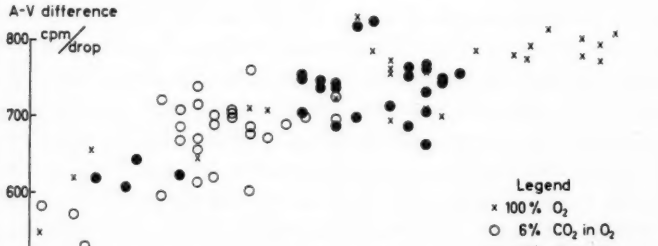

Fig. 38. Rabbit. 3.0 kg. Nembutal-chloralose. At the beginning of the experiment, 200 μC I^{131}. The graph shows the A-V difference of I^{131} in relation to thyroid blood flow in three consecutive tests of the effect of 2 μg adrenaline intravenously when the rabbit was breathing pure oxygen (x), 6 % carbon dioxide in oxygen (open circles) or 8 % oxygen in nitrogen (filled circles), respectively. Original record of thyroid blood flow is shown in Fig. 21.

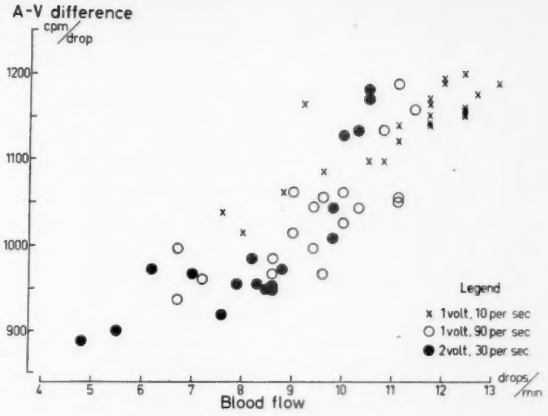

Fig. 39. Rabbit. 2.8 kg. Nembutal. Forty min before the record, 200 μC I^{131}. The graph is drawn similarly to that of Fig. 38 but shows three tests of the effect of electrical stimulation of the ipsilateral cervical sympathetic trunk, stimulus strength and frequency given in lower right corner.

thyroid while the flow was reduced from 40 to 20 drops per min. In another experiment clamping one carotid artery caused a fall in the A–V difference whereas clamping the other was followed by an increase, probably indicating that the extra-thyroidal vascular beds had not been successfully eliminated and possessed somewhat different arterial supplies. There is in fact a considerable asymmetry in the vascular anatomy of this region of rabbits and cats.

The graphical reproduction of the relation between the rate of thyroid blood flow and the A–V difference of radioiodine plotted as discrete points, has in many experiments been used as a simple method of studying the effects of the stimuli. By comparing the relative spread of each set of values, such a graph also provides an immediate and direct index of the statistical significance of the measurements. Three experiments of this kind will be discussed.

One of them, shown in Fig. 38, is a study of the influence of the tension of blood gases on the A–V difference during the adrenaline test. Two micrograms of adrenaline were injected intravenously each

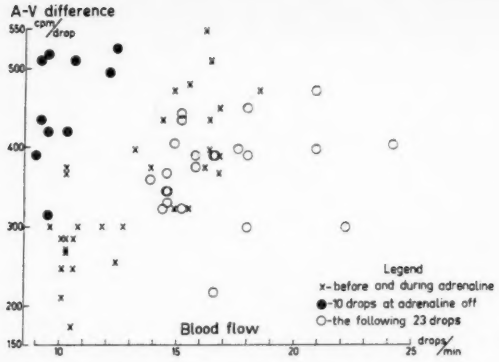

Fig. 40. Rabbit. 2.6 kg. Nembutal. 100 μC I^{131} 90 min, and 4 U. S. P. units TSH 70 min before the record. The graph illustrates the relation between A–V difference and blood flow in an experiment in which adrenaline was slowly infused into the femoral vein. Original records in Fig. 41. Crosses indicate values obtained before and during infusion, filled circles the first 10 drops after infusion ended, and the open circles, the next 23 drops. Note the sharp change in the first interval after infusion ended. Further explanation in text.

time. The animal was breathing pure oxygen (x), 6 per cent carbon dioxide in oxygen (open circles) or 8 per cent oxygen in nitrogen (filled circles). Within the limits of experimental error all the values obtained seem to be distributed along the same curve. From this result, it is assumed that changes of tension of the blood gases have no direct influence on thyroid uptake, and that the effect of adrenaline on the uptake of iodine ist not caused by oxygen lack or carbon dioxide accumulation in the gland during the vasoconstriction. There is, however, an evident effect of the change of the systemic concentrations of oxygen and carbon dioxide on the thyroid blood flow since the points are not randomly distributed along the curve. Both oxygen lack and carbon dioxide excess have increased the vasomotor tone and thereby also apparently reduced the rate of uptake of iodine.

The second example of a relation between flow and A–V difference is illustrated in Fig. 39 obtained from a rabbit in which the influence of electrical stimulation of the cervical sympathetic was

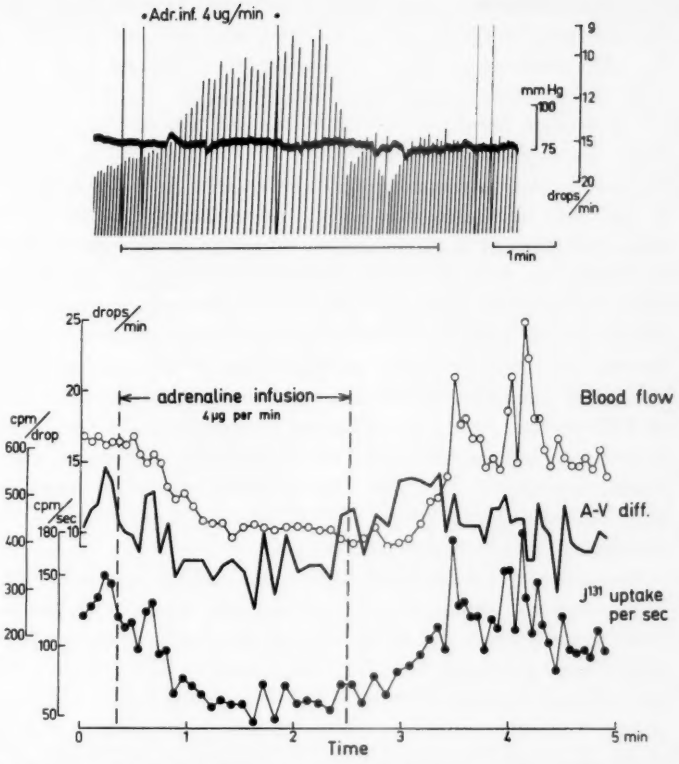

Fig. 41. Original records of the experiment of Fig. 40. *Upper graph:* Femoral blood pressure and thyroid blood flow from the right lobe. *Lower graph:* Blood flow, A–V difference of I^{131} and rate of uptake. Below upper graph, a horizontal bar shows time relation to the lower graph. The slow infusion of adrenaline is indicated. Irregularities in blood flow 1 min after cessation of the infusion coincided with 'arousal' activity in the behaviour of the animal.

studied. The responses to three kinds of stimulation (*cf.* "Legend," inset) are reproduced. Although quite different vasomotor responses were obtained, all the plotted values probably belong to the same function.

A few exceptions to this simple relationship were observed. The most apparent of them, still unexplained, was regularly obtained by the administration of adrenaline to animals pretreated with large doses of TSH. This is illustrated in Fig. 40 and 41. Fig. 40 is a graph of the relation between A–V difference and rate of flow, Fig. 41 shows the original flow and pressure (top record) and flow, A–V difference and rate of uptake of I^{131} below. The A–V difference fell in the usual way during the initial vasoconstriction. At the end of adrenaline infusion in this example, and earlier in the case of single injections, the A–V difference transiently increased to unexpected values before there were any signs of reduced vasomotor tone. This change in the response to adrenaline was elicited as soon as a few minutes after the intravenous administration of TSH and could still be elicited and measured two hours afterwards. Evidently this effect of TSH on the response to adrenaline has not the same time course as the well known effect of TSH on iodine accumulation whose latency is at least a few hours. The change in response to adrenaline is in fact the earliest effect of TSH on the thyroid so far known. For its relation to the hormone secretion, see p. 61.

In one rabbit a marked reduction of the A–V difference occurred after adrenaline although the net effect of the hormone on the systemic blood pressure and on the thyroid vascular resistance produced little change in thyroid blood flow. This is shown in Fig. 42 in which only the venous concentration of iodine is reproduced in the lower graph. The concentration of iodine in the blood (which, as stated above is a mirror image of the A–V difference) is reproduced in arbitrary units, the venous level immediately before adrenaline taken as 100 per cent. Two isotopes of iodine were tested simultaneously, the I^{132} having been administered 70 min earlier than the I^{131} which was injected as late as 10 min before adrenaline was given. The upper graph reproduces the original registration of flow and pressure and immediately below this, a horizontal line indicates the period of recording the venous radioiodine of the lower graph. Note the similarity in behaviour of the two isotopes of iodine although they had been injected at different times.

Another exception to the simple relationship between blood flow and A–V difference was obtained with antidiuretic hormone (ADH).

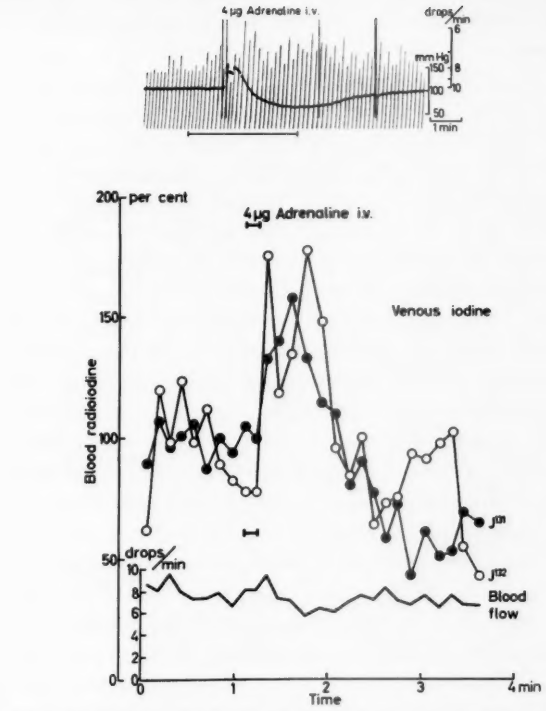

Fig. 42. Rabbit 2.8 kg. Nembutal. I^{132} 70 min and I^{131} 10 min before the record in the figure. *Upper graph:* Record of femoral blood pressure and flow from left thyroid lobe. *Lower graph:* Venous concentration of the two radioactive isotopes of iodine plotted in arbitrary units, the basal level chosen as 100 %. Below venous iodine, the blood flow is reproduced as reciprocal values of time intervals between blood drops. Below upper graph a horizontal bar shows the time relation to lower graph. As indicated, 4 µg adrenaline intravenously.

After large doses, the A–V difference of iodine was less reduced than one would have expected from the degree of vasoconstriction, and was not exactly related to blood flow. This may be seen in the lower half of Fig. 43 from a cat under Nembutal. The minimum A–V difference (heavy lines without circles) appeared 40 sec before the

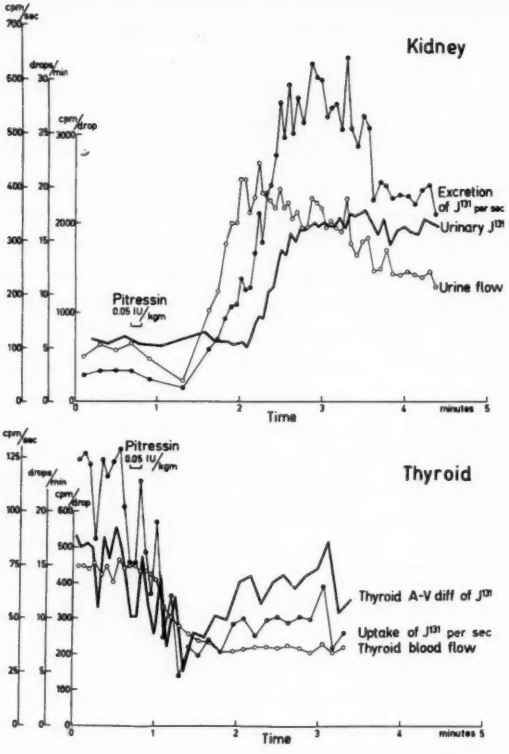

Fig. 43. Cat. 4.9 kg. Nembutal. Simultaneous measurement of urinary excretion of radioiodine and thyroid uptake. 200 μC I^{131} administered at the beginning of the experiment. *Upper graph:* Kidney functions, *lower graph:* thyroid activity, simultaneously. Thus, ***thin lines between open circles*** represent urine flow and thyroid blood flow, ***heavy lines*** concentration of I^{131} in urine and thyroid A–V difference of I^{131}, and ***thin lines with filled circles*** show rate of I^{131} excretion by the kidney and rate of I^{131} uptake by the thyroid. When indicated, 50 mU antidiuretic hormone per kg body weight intravenously. Note rapid reduction of I^{131} uptake by the thyroid and increased urinary excretion. The two events were not closely related in time and apparently not exactly related to blood flow or urine flow either.

flow (thin line with open circles) had reached its lowest value. The A–V difference then slowly increased although the flow remained at a low rate. In the same figure is reproduced the simultaneously recorded activity in the left kidney, measured in a manner similar to the estimation of thyroid activity. A thin nylon tubing was introduced through the ureter and the drops of urine were also collected on the filter paper. In the calculation corrections were made for the urinary volume in the ureteral catheter and in the kidney from the site of iodine excretion to the proximal end of the catheter. The figure shows a transient reduction of the urine flow followed by the well known increase that is obtained by ADH in Nembutal anaesthesia (MAGNUS and SCHÄFER, 1901; BLAKE, 1957). The urinary concentration of I^{131} was constant during the first 85 sec and then rapidly increased to three times its original level and remained high throughout the measuring period. The apparent difference between the mechanism of I^{131} uptake in the thyroid and I^{131} excretion through the kidney is well known (RIGGS, 1952 for references). The experiment is only described here to present a quantitative comparison of the effect of ADH on the two organs of the experimental animal.

Sometimes a very steep rise in A–V difference occurred when the increase of flow rate was comparatively small, as shown in Fig. 44. In this experiment, brom-lysergic acid diethylamide, BOL–148, caused a very marked vasodilatation. The A–V difference reached its highest level while rate of flow had only increased from 40 to 45 drops per min. It is not yet clear whether this increase follows the general relationship described above or whether this particular drug, famous for its powerful inhibitory action upon serotonin, also has a direct potentiating effect on the uptake of iodine. Lysergic acid diethylamide, another powerful serotonin antagonist, did not show such a property but on the contrary gave a slightly reduced A–V difference related to a concomitant reduction of the blood flow.

Anaesthetics

All the anaesthetics tested followed the general rule of the relation of flow and A–V difference. Thus the immediate effect of the barbiturates was an i n c r e a s e in the uptake of iodine since they all

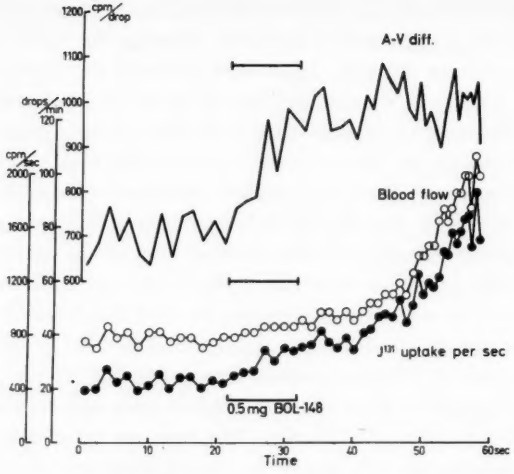

Fig. 44. Rabbit. 2.7 kg. Nembutal. I^{131} at the beginning of the experiment. *From above downwards:* A–V difference, blood flow and rate of uptake of I^{131}. When indicated, 0.5 mg Brom-lysergic acid diethylamide ('BOL-148') intravenously. Note rapid increase in A–V difference when flow increased from 40 to 45 drops per min.

elicited a transient vasodilatation. Chloralose, on the other hand, transiently reduced the uptake, in good agreement with the vasoconstriction that appeared. There is, however, no doubt that the different anaesthetics influenced thyroid activity more profoundly than was indicated by the small and transient effect on the uptake. It will be shown below that they all could reduce the rate at which trapped iodine is utilized (p. 55–58).

Thiouracil and mercaptobenzimidazol

As in the case of the anaesthetics, no immediate effect was observable in the general level of the thyroid iodine clearance after administration of propyl thiouracil or dimethylolmercaptobenzimidazol. Both elicited a transient vasodilatation, with increased A–V difference of I^{131} as a consequence. If, however, mercaptobenzimid-

azole was used as a basal anaesthetic (as described on p. 39) the A–V difference of iodine was markedly reduced when measurements were begun a few hours after the onset of the 'Matinol anaesthesia.'

Discharge of labelled iodine from the thyroid

Thiocyanate and large doses of unlabelled sodium iodide were used in order to release iodine trapped by the gland but not yet utilized. This has been done in the rabbit, *e. g.* by BROWN-GRANT and GIBSON (1955) who showed that 200 mg potassium thiocyanate reduced or arrested the uptake of radioiodine in the normal (unanaesthetized) animal but caused the release of all trapped radioiodine in rabbits pretreated with thiouracil. It has also been shown that one gram of potassium thiocyanate can cause a complete discharge of radioiodine accumulated by the thyroid of a goiterous cretin (STANBURY and HEDGE, 1950). This property of thiocyanate is shared by other monovalent ions (WYNGAARDEN, WRIGHT and WAYS, 1952). Fig. 45 shows the effect of 300 mg potassium thiocyanate on the venous (x) and arterial (– – –) concentration of radioiodine of a very lightly anaesthetized cat 60 min after the administration of the tracer. There is an immediate increase in thyroid venous concentration even beyond the arterial values which means that iodine is discharged from the gland. However, outward diffusion is still less than was the inward diffusion just before the injection of thiocyanate, indicating that this animal behaved similarly to those of Brown-Grant and Gibson which had not been pretreated with thiouracil, *i. e.* the iodine trapped by the thyroid had rapidly been utilized for synthesis of hormones or precursors to them. Below the blood concentration curves there is an inset of the original record of flow and of blood pressure, showing the transient vasodilatation that generally occurred after thiocyanate.

Although there is a concentration gradient of iodide from inside to outside the thyroid cells and follicles, the iodide is freely diffusible in both directions. Therefore a large dose of one isotope of iodine, when injected into the blood stream, will always replace a portion of another isotope already taken up by thyroid. Thus a large dose of I^{127} can cause a discharge of I^{131} already taken up by the gland but stored in it as iodide. This is shown in Figs. 46 and 47. The former

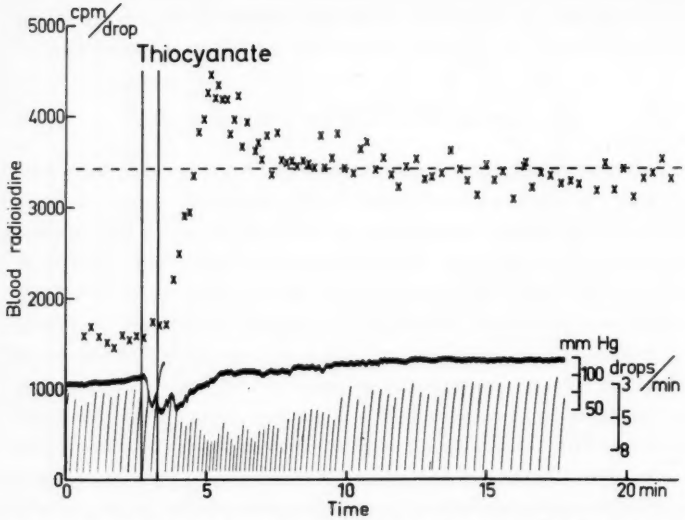

Fig. 45. Cat. 3.8 kg. Light Nembutal. I^{131} 60 min before the record. Thyroid venous I^{131} (x) and average arterial concentration (– – –). Original records of femoral blood pressure and thyroid blood flow in lower part of the figure. Between thick vertical line, 300 mg sodium thiocyanate intravenously.

shows the effect of 5 and 50 mg NaI^{127} injected intravenously into a rabbit 120 min and 150 min respectively after the administration of I^{131}. The smaller dose temporarily reduced the A–V difference of I^{131} while the larger dose changed the A–V difference to negative values *i. e.* changed the net direction of diffusion of I^{131} from inwards to outwards. Note the regularly occurring minima of the two curves showing that it takes some time before the freshly injected iodine is evenly distributed in the blood.

Fig. 47 illustrates the effect of 200 mg of unlabelled sodium iodide (NaI^{127}) on the relative concentrations of I^{131} and I^{132}. The I^{132} was given 85 min before the I^{131} and 25 min before the I^{127}. Thus a larger quantity of I^{132} had probably diffused into the gland than of I^{131}. More of the I^{132} left the gland per unit time to judge by the relative values of negative A–V difference.

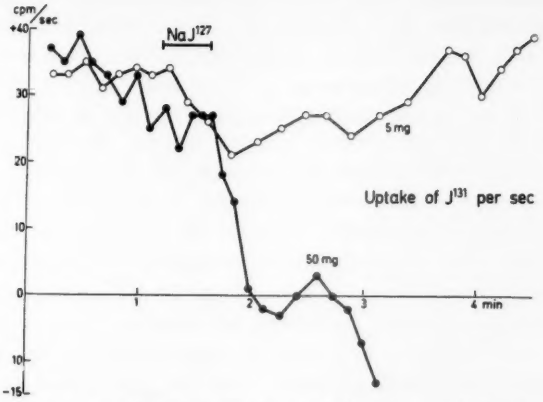

Fig. 46. Rabbit. 2.9 kg. Nembutal. I^{131} 120 min before the record. Two consecutive periods of measuring rate of uptake of I^{131} in left thyroid lobe are drawn to the same scale, related in time by the moment of intravenous injection of the large doses of NaI^{127}. The injection of the 5 mg dose was made 30 min before the 50 mg dose. Negative values of rate of uptake indicate discharge of accumulated iodide.

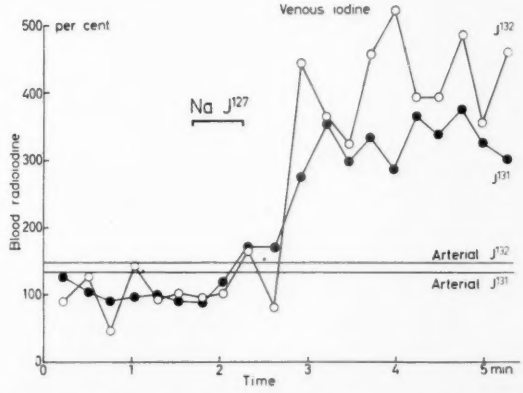

Fig. 47. The rabbit of Fig. 42. (2.8 kg. Nembutal), 15 min after the period of measurement of that figure. Arterial and venous concentration of the two isotopes of iodine in arbitrary units with thyroid venous level before mark chosen as 100 %. As indicated, 200 mg NaI^{127} injected intravenously caused an immediate discharge of radioiodine previously accumulated by the thyroid gland.

In general, more iodine diffused out of the gland than one would have expected from the values for animals not treated with thiouracil as given by BROWN-GRANT and GIBSON (1955). This is in good agreement with the observation by WASE and FOSTER (1956) that some barbiturates also inhibit the organic binding of iodine by the thyroid.

The release of labelled hormone

In 28 rabbits and 2 cats the rate of release of labelled thyroid hormone was studied with the same method used for the measurement of the uptake of radioiodine. The rate of secretion was also measured in two cats according to the method of BROWN-GRANT, C. VON EULER, HARRIS and REICHLIN (1954). However, the cats were anaesthetized with ether during the measurements since they were not trained to lie in a hammock. Twenty μC of carrier-free radioiodine were injected 48 hours before the first test of radioactivity. The results are shown in Fig. 48 where the neck radioactivity is plotted in counts per min on a logarithmic scale against time in hours on a linear scale with proper corrections for the physical decay of the isotope. The rate of release found was somewhat lower than the rate usually seen in rabbits. The cats had, however, been fed on a diet containing Baltic herring and the total content of iodine in thyroid gland and blood was not measured.

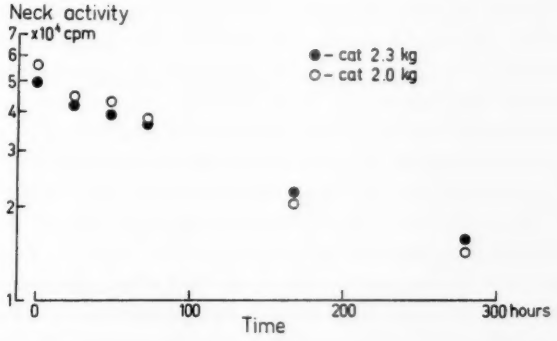

Fig. 48. Changes in radioactivity in the thyroid region of 2 cats given a tracer dose of I^{131} 48 hours before zero time. Measurement according to BROWN-GRANT *et al.* (1954). Semi-logarithmic plot with correction for the physical decay of the I^{131}.

The animals used for estimations of the rate of release of labelled thyroid hormones from determinations of the A–V difference of protein bound iodine were pretreated with 50 to 200 μC of carrier-free I^{131} one to three days before the acute experiment. Some of the animals were simultaneously given 5 U. S. P. units of TSH. This TSH treatment had three favourable consequences: i) it initially facilitated the release of unlabelled hormone, ii) it accelerated the uptake of the tracer, and iii) the initially released hormone inhibited endogenous TSH from the pituitary (*cf.* BROWN-GRANT, 1957) so that only a small portion of the labelled thyroid hormone was lost before the acute experiment. Pretreatment with TSH was only done in a small portion of the animals since it evidently disturbed the normal pituitary-thyroid relationship to a degree that could not be quantitatively determined.

The A–V difference over the thyroid was generally very near zero or slightly negative when the thyroid vein was cannulated one to three days after the administration of the radioiodine, provided that the tracer was found in the protein precipitable fraction of the plasma. Large amounts of unlabelled sodium iodide (NaI^{127}) which by simple dilution reduced to zero the uptake of small amounts of NaI^{131} still circulating in the blood, sometimes caused a significant negative A–V difference. This was regarded as a relative measure of the thyroid hormone secretion. For simplicity, the difference between the arterial and venous concentration will be described below in terms of V–A difference in all experiments in which the hormone secretion is determined. The V–A difference is opposite in sign to the A–V difference and so will be positive when the rate of secretion of hormonal radioiodine is higher than the simultaneously occurring uptake of radioiodine. By design, the uptake was zero because of previous administration of NaI^{127} in doses up to 1 000 mg; a few exceptions are mentioned below.

The V–A difference was particularly high when the operative procedure had been completed quickly and measurements were started one to two hours after the introduction of anaesthesia. The V–A difference invariably declined and, as a rule, was zero within three hours after the beginning of the operation. Positive V–A differences sometimes then reappeared even in animals which were not treated in

any particular way. This effect, interpreted as due to reappearing pituitary secretion of TSH, was slow and variable and it was not possible to obtain any correlation between electrical stimulation of the central nervous system and the thyroid response.

In cases where the rate of release of hormone was zero, electrical stimulation of thyroid nerves, administration of hormones such as adrenaline and acetylcholine, or injections of drugs never evoked any secretory activity. In cases when the V–A difference indicated that there was some th- -oid activity small effects could by observed after many of these stimuli. These effects will be described later on since they could be increased by exogenous TSH. Other ineffective stimulations were slow intravenous infusion of adrenaline and prolonged stimulation of the cervical sympathetics. In the past, the cervical sympathetics have often been credited with the ability to elicit thyroid hormone secretion (references in "Appendix"). In one experiment the thyroid veins from both halves of the gland were cannulated and the sympathetic on one side stimulated for 80 min at a stimulus strength that caused only a transient vasoconstriction on the stimulated side. The V–A difference slowly increased on both sides but not more than was observed in control animals which had not been subjected to any tests. Consequently, in the present work, no support for the existence of secretory nerves in a strict sense has been obtained.

Thyroid stimulating hormone (TSH)

It has already been mentioned that a slight transitory release of thyroid hormone was obtained early in many experiments and that some activity also appeared late in prolonged experiments and that these effects were attributed to endogenous TSH. When exogenous thyrotropic hormone was administered, a marked V–A difference developed with a latency of 5 to 30 min and a peak value after 30 to 60 min in the rabbits, and after about two hours in the cats, provided that the rate of thyroid blood flow was constant. This secretion was probably considerable even in absolute values since the V–A difference was sometimes higher than the A–V difference observed during the measurements of the uptake of a dose of the same size

as that used for labelling the thyroid hormone in the release experiments. This was particularly the case if the uptake of the tracer in animals intended for release measurements was accelerated by simultaneously administered TSH.

In a few experiments the thyrotropic hormone was administered during slow infusion of adrenaline or during electrical stimulation of the cervical sympathetic trunk. The thyroid hormone secretion that was obtained in response to TSH administered in this way was unusually small. Additional TSH, given as a control whithout concomitant stimulation one hour after the first injection, had greater effect. These experiments suggest that the circulatory state of the thyroid was of importance for the thyroid response to the pituitary stimulating hormone but further experimentation is needed to substantiate this finding.

Influence of thyroid blood flow

The V–A difference increased when blood flow was slow and decreased when the rate of flow increased. This could occur in response both to vasomotor changes and to changes in the arterial blood pressure. As shown in Fig. 49 increased rate of intravenous infusion caused a slight rise of the arterial blood pressure and a marked increase of the thyroid blood flow (broken line with open circles). The V–A difference of labelled hormone (continuous line with open circles) decreased. The rate of release (filled circles), calculated from the values of flow and V–A difference, was found to remain constant throughout the period of measurement reproduced in the figure.

Sympathetic stimulation

Electrical stimulation of the cervical portion of the sympathetic trunk invariably caused vasoconstriction and elicited a small but probably significant increase in the rate of secretion of labelled hormone induced by TSH. However, further experimentation must be done to determine whether the sympathetic nerves also can inhibit the release. Such inhibition will be shown below to occur after large doses of adrenaline and noradrenaline (for the effects of adrenaline and reflex activities, see below).

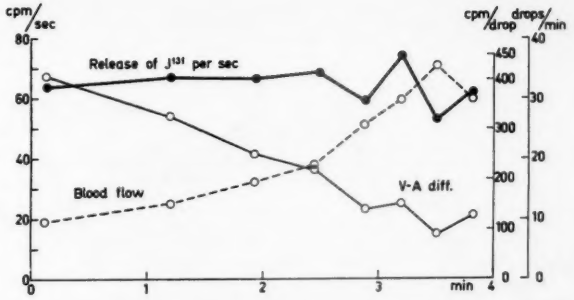

Fig. 49. Rabbit. 2.7 kg. Nembutal. 150 μC I^{131} 48 hours before, 4 U. S. P. units TSH at the beginning of acute experiment. Large dose of NaI^{127} administered to eliminate uptake of I^{131} as described in the text. *Open circles, dashed line,* blood flow of left thyroid lobe (calibration to the right in drops per min); *open circles, continous line,* V–A difference of I^{131}, most of which was organically bound (calibration to the right in cpm/drop); and *filled circles, continuous line,* rate of release of I^{131} from the thyroid as a measure of thyroid hormone secretion (calibration on the left in cpm/sec). During the period shown, the rate of intravenous re-infusion was increased with increased rate of thyroid blood flow as a consequence. Note decreased V–A difference of I^{131} when flow increased but constant rate of release of I^{131}.

Since the error in measurements of V–A difference increases as V–A difference falls, and this error is further enlarged when V–A difference is multiplied by a high rate of flow, then the error in rates of release will increase as flow increases. This may explain the fluctuation in the rate of release curve between curve between 3 and 4 min.

Superior laryngeal nerve

Electrical stimulation of the superior laryngeal nerve very often elicited thyroid vasodilatation but only rarely facilitated the effect of TSH. Consequently, there seemed to be little or no correlation between the effect on blood flow and the action on the secretory mechanism. The V–A difference could decrease, remain unaltered or increase. One of the largest effects observed is reproduced in Fig. 50 from a rabbit in which the stimulation (2 volts 50 per sec) caused a two-fold increase in the rate of release of several minutes duration but with a latency of 45 sec from the onset of the stimulation. The vasomotor activity showed a similar response.

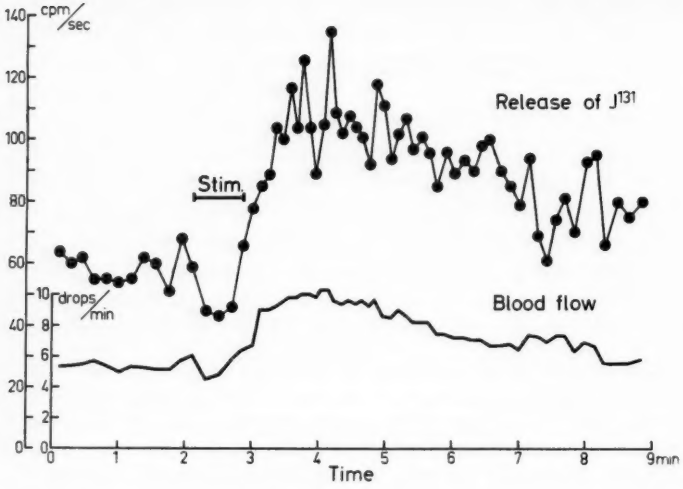

Fig. 50. Rabbit. 3.0 kg. Nembutal. 150 μC I^{131} 24 hours before, 4 U. S. P. units of TSH at the beginning of the experiment. Rate of release of hormonal I^{131} (filled circles) and thyroid blood flow (continuous line). As indicated, electrical stimulation of the peripheral end of the cut superior laryngeal nerve (2 volts, 50 per sec).

Reflexes

More successful than was direct stimulation were attempts to influence thyroid activity by reflexes in which either the laryngeal nerve or the sympathetic trunk was found to be the efferent pathway. Thus, a reflex set up in the central end of the cut vagus nerve and mediated by the laryngeal nerve could result in a very pronounced effect both on flow and on secretion (Fig. 51). The thyroid response shown in Fig. 51 is more impressive than may be evident from the figures of the rate of release given below the record of the blood flow, since it was very unusual at such a low resting level of secretion to find a significant effect so early after TSH.

A facilitatory reflex mediated by thyroid nerves has also been observed after the injection of brom-lysergic acid diethylamide (Bol-148)

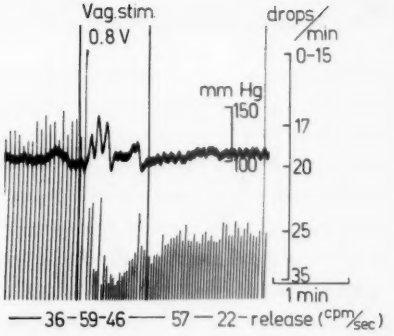

Fig. 51. Rabbit. 3.0 kg. Nembutal. I^{131} 48 hours before, TSH at the beginning of the experiment. Femoral blood pressure and blood flow from left thyroid lobe. Below flow record, rate of release of hormonal I^{131} indicated by figures. Between heavy vertical lines, electrical stimulation of the cranial end of the cut vagus nerve on the left side (0.8 volts, 100 per sec). *Cf.* Fig. 8 which is taken from the same experiment.

as is shown in Fig. 52. In this figure the activity in both halves of the thyroid is registered simultaneously, one half being completely denervated (open circles). The drug had a weak vasodilatatory effect on both sides but promptly increased the rate of secretion in the intact half by about 100 per cent although the duration of the effect was less than a minute. Similar effects have also been observed during the 'arousal reaction', provided that the changes of activity were recorded from that half of the gland which had intact nerves. The site of action of BOL-148 has not yet been investigated. The effect may be secondary to a peripheral action of the drug seen *e. g.* in the fall in systemic blood pressure or it may be a result of direct stimulation of the brain. The closely related hallucinogen, lysergic acid diethylamide, LSD-25, did not facilitate thyroid hormone secretion, nor did it cause vasodilatation.

In general, nervous influence on the thyroid both in 'spontaneous arousal' and in experimental reflexes had a much more powerful effect than any dose of adrenaline or noradrenaline injected into the blood stream or released from the adrenals.

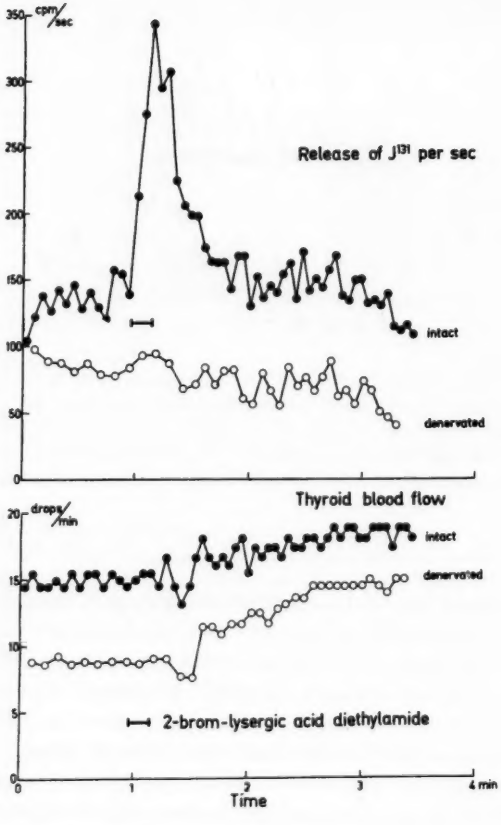

Fig. 52. Rabbit. 3.0 kg. Nembutal. Hormone secretion induced by exogenous TSH. *Upper graph:* Rate of release of hormonal I^{131} from intact and acutely denervated halves of the thyroid gland respectively. *Lower graph:* Rate of venous outflow from the same halves. Brom-lysergic acid diethylamide ('BOL-148') increased rate of release by more than a hundred per cent in the intact half of the gland. Blood flow increased slightly in both halves although blood pressure (not reproduced in the figure) showed a slight transient fall.

Fig. 53 demonstrates the effect of two micrograms of adrenaline on a rabbit which had been treated with a large dose of TSH half an hour earlier. The uppermost records *(A)* show the most common effect on blood pressure and blood flow, the latter with an initial vasoconstriction followed by vasodilatation. Below that record a horizontal bar marks the period during which blood samples were taken for estimating the radioactivity plotted in *C* and *D*. For simplicity, the blood flow is also graphically reproduced in *B*, as the reciprocal value of the intervals between successive drops. In the series of drops of blood on the filter paper which represented the blood that had passed through the thyroid shortly after the intravenous injection of the adrenaline, the radioactivity increased considerably and then decreased slightly below the resting level *(C)*. If the value of the arterial concentration of radioiodine was subtracted from the venous values and the remainder (V–A) multiplied by the rate of flow, an estimate of the rate of release was obtained. This is graphically demonstrated in *D* and shows that there is also an increase in the r a t e of secretion of labelled hormones but if compared with *C* the peak has moved slightly to the right to a point approximately corresponding to the change from vasoconstriction to dilatation. Although similar results were frequently obtained, on other occasions adrenaline was completely without effect, as in Fig. 54 from a rabbit in which both the thyroid veins were cannulated. The blood flow record at the bottom of the figure exhibits the usual vasoconstriction which is reflected as an increased V–A difference of radioiodine shown in the middle records. However, the calculation of the release of hormone per unit time (uppermost tracings) revealed that, within the limits of the experimental error, there was no change.

Being a function of the interval between successive blood drops, the resolution in time was generally rather poor in experiments with vasoconstriction. As it was desirable to know whether the peak values of the rate of secretion after adrenaline (seen *e. g.* in Fig. 53) are related to the vasoconstriction or to the dilatation, the adrenaline was also administered by slow infusion into the femoral vein. Such an experi-

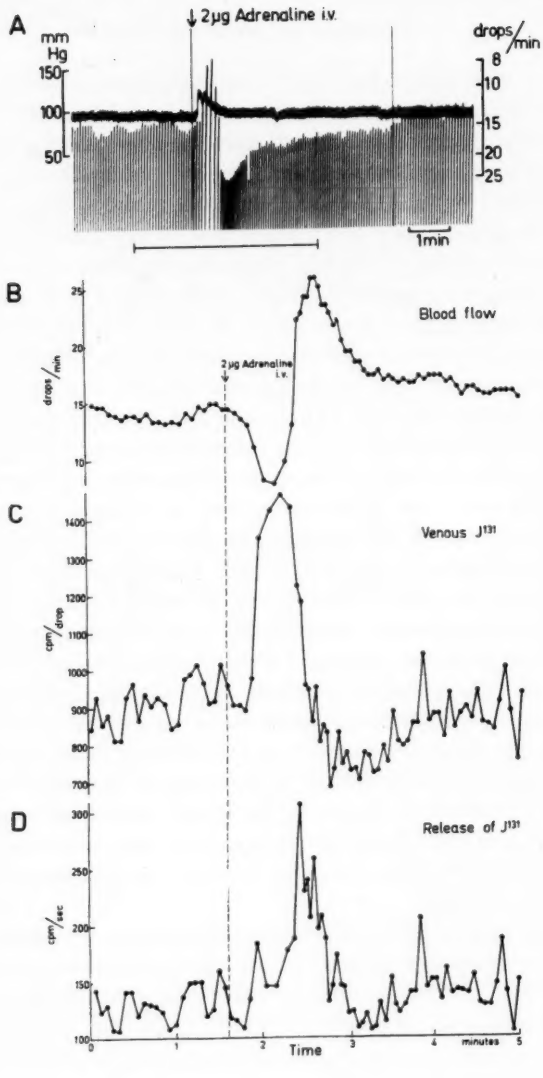

Fig. 53. legend on p. 69.

ment is illustrated in Fig. 55; the upper part is a reproduction of the original tracings of flow and blood pressure. In the lower part of the figure the V–A difference and the rate of secretion are drawn to the same time scale as the upper part of the figure. The period of infusion is given above the figure. Immediately after the onset of infusion, the V–A difference increased considerably until a steady state was reached. The rate of secretion was initially depressed to about 50 per cent of the resting level but returned to it after about two min. At the end of the infusion a transient increase in the V–A difference appeared although the blood flow increased within 10 sec at the end of the infusion. This peak was therefore even more marked in the graph representing rate of release, showing an increase of about 300 per cent at the maximum. The 'off-effect' was not closely related to thyroid blood flow, since it appeared even in those experiments in which flow was constant because of a favourable balance between arterial blood pressure and thyroid vasomotor resistance. As large doses of adrenaline also have an inhibitory action *e. g.* on some metabolic processes (U. S. VON EULER, 1954), a possible explanation of the 'off-effect' is that the hormone secretion is inhibited by high concentrations and stimulated only by low concentrations of adrenaline. This hypothesis is supported by the observation that a relatively large dose of adrenaline administered by a direct arterial route produces a marked reduction of the rate of hormone release followed by a late increase (see Fig. 59 *A*). Noradrenaline was found to have the same effects as adrenaline but it was about ten times weaker. This explains the paradoxical result of Fig. 59 *B* where noradrenaline also injected by a direct arterial route, had an effect on the thyroid with shorter latency than that of adrenaline. Both the hormones elicited approximately the same moderate degree of vasoconstriction in this experiment.

Fig. 53. Rabbit. 2.3 kg. Nembutal-chloralose. Secretion of labelled thyroid hormone induced by exogenous TSH. *Records from above:* Femoral blood pressure and time intervals between thyroid blood drops *(A);* thyroid blood flow calculated from reciprocal values of distance between drops on the filter paper *(B);* thyroid venous I^{131} in cpm/drop *(C);* and rate of thyroid release of I^{131} in cpm/sec *(D).* Time relation between *A* and *B – D* shown by horizontal bar below *A.* When indicated, 2 μg adrenaline intravenously. Venous I^{131} and, to some extent, rate of release of labelled hormone increased after the injection.

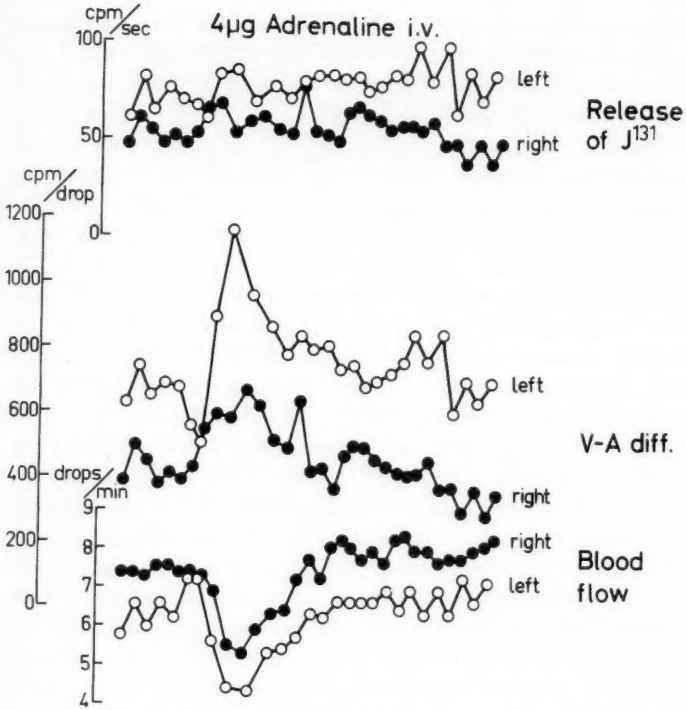

Fig. 54. Rabbit. 3.0 kg. Nembutal. Thyroid hormones labelled with I^{131}. Hormone secretion induced by exogenous TSH. *Records from above downwards in pairs:* Rate of release of hormonal I^{131}, V–A difference and thyroid blood flow. *Open circles* from left, *filled circles* from right thyroid lobe. At time one min, 4 μg adrenaline intravenously. Note thyroid vasoconstriction with compensatory increase in V–A difference. Rate of release unchanged.

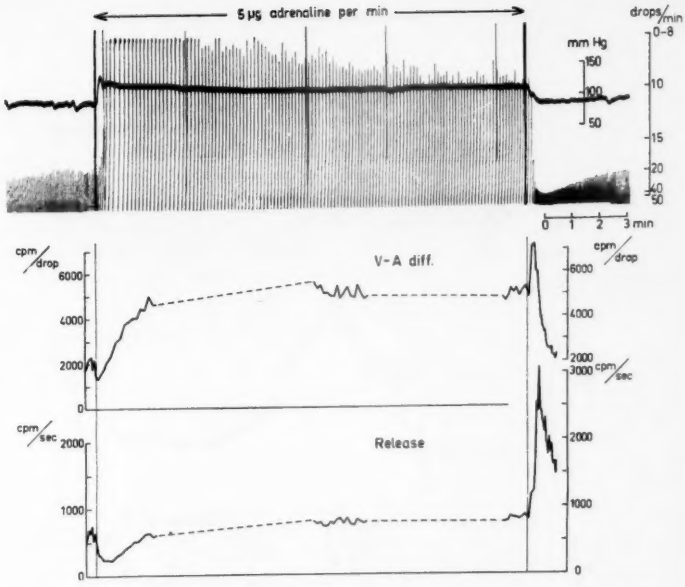

Fig. 55. Rabbit. 2.3 kg. Nembutal-chloralose. Thyroid hormones labelled with I^{131}. Hormone secretion induced by exogenous TSH. *Records from above downwards on the same time scale:* Femoral blood pressure, blood flow from left thyroid lobe, V–A difference of I^{131}, and rate of release of labelled hormones. As indicated, adrenaline slowly infused (5 µg per min). The V–A difference rapidly rose to a level that fully compensated for the reduction of thyroid blood flow so that rate of release remained uninfluenced except for the initial minute when there was a slight reduction. However, at the cessation of the adrenaline infusion the V–A difference transiently increased although the vasoconstriction diminished. The result was that the rate of release showed a considerable 'off-effect' while the hormone secretion transiently rose to 4 to 5 times the initial level.

Acetylcholine

The injection of acetylcholine invariably increased the rate of hormone secretion induced by TSH. The effect was more striking than that of adrenaline and no sign of inhibition could be obtained even by very large doses. A period of decreased secretion generally followed

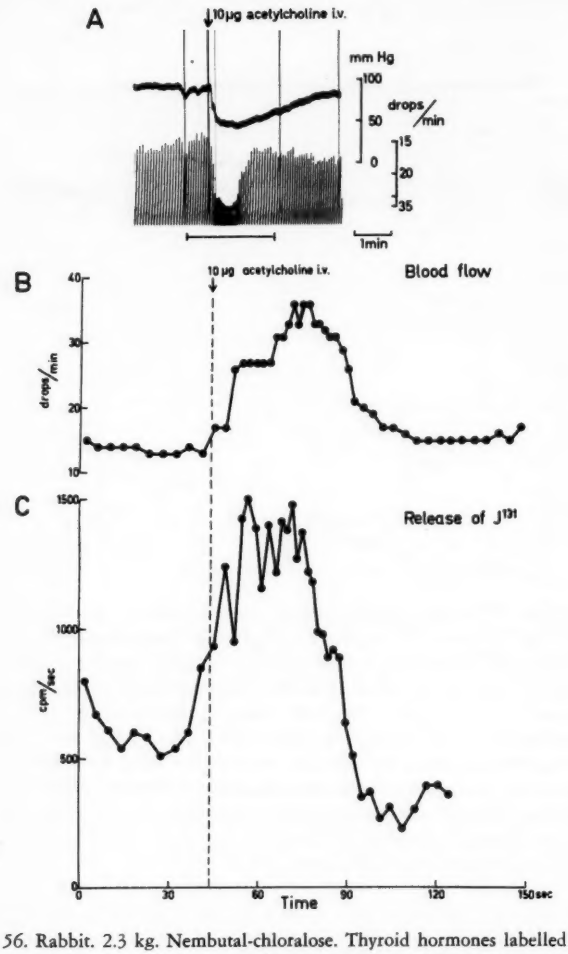

Fig. 56. Rabbit. 2.3 kg. Nembutal-chloralose. Thyroid hormones labelled with I^{131}. Secretion induced by TSH. *From above downwards:* Femoral blood pressure and blood flow from left thyroid lobe *(A)*, thyroid blood flow as reciprocal value of time interval between drops *(B)* and rate of release of labelled hormone *(C)*. Below flow record in *A* a horizontal bar indicates time between beginning and end of *C*. At vertical line below arrow in *A* and at dashed line in *B* and *C*, 10 µg acetylcholine intravenously.

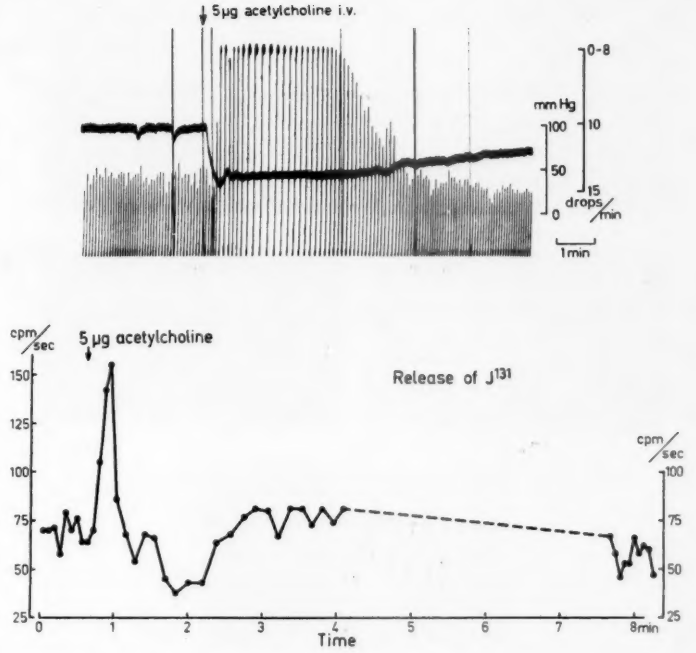

Fig. 57. Rabbit. 2.4 kg. Nembutal. Thyroid hormones labelled with I^{131}. Hormone secretion induced by exogenous TSH. *Upper graph:* Original record of femoral blood pressure and blood flow from left thyroid lobe. *Lower graph:* Rate of release of labelled hormone during the period between first and fifth vertical lines in upper graph. As indicated, 5 µg acetylcholine intravenously. During period of dashed line in lower graph, venous and arterial blood samples were drawn to ascertain the value of the A–V difference.

the stimulatory effect. Fig. 56 shows a two-fold increase in the release of labelled hormone after 10 micrograms of acetylcholine was injected intravenously into a rabbit. In this case the thyroid blood flow increased from 15 to 35 drops per min despite a fall in blood pressure from 95 to 50 mm Hg. The period of increased rate of secretion had a duration of 45 sec and was succeeded by a period of reduced rate in which the release of hormonal radioiodine was diminished to about 50

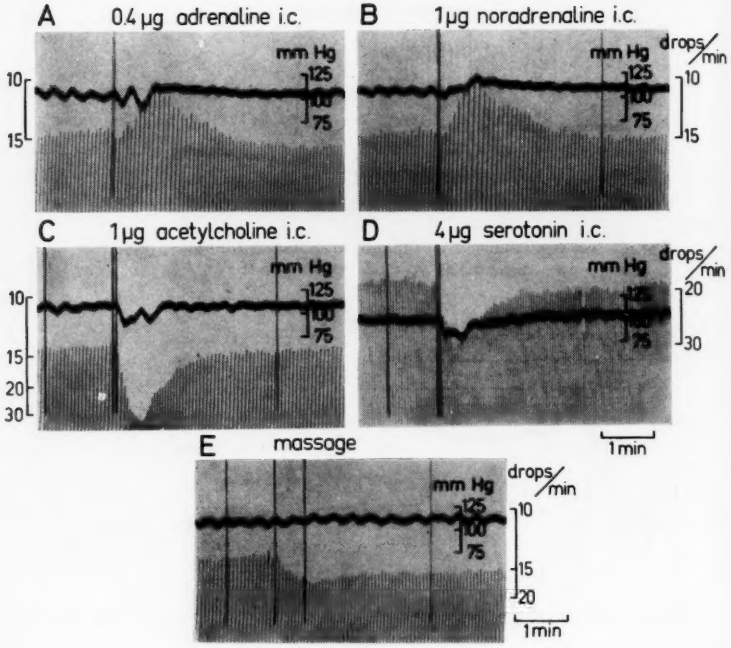

Fig. 58. Cat. 4.7 kg. Nembutal. Hormones labelled with I^{131}. Secretion induced by TSH. Five original records of femoral blood pressure and thyroid blood flow from left thyroid lobe. Testing procedures indicated above each record. Full description in the text (*i. c.* = injection into the common carotid artery).

per cent of the resting level. The close correlation between blood flow and secretion of hormone was a mere coincidence. Fig. 57 from another rabbit illustrates an example in which the fall in the systemic blood pressure was so great in relation to the vasodilatation in the thyroid that the blood flow was reduced. Nevertheless, the release curve showed a transient increase of about 100 per cent before exhaustion occurred. Note that the period of decreased secretion was much shorter than the period of reduced thyroid blood flow.

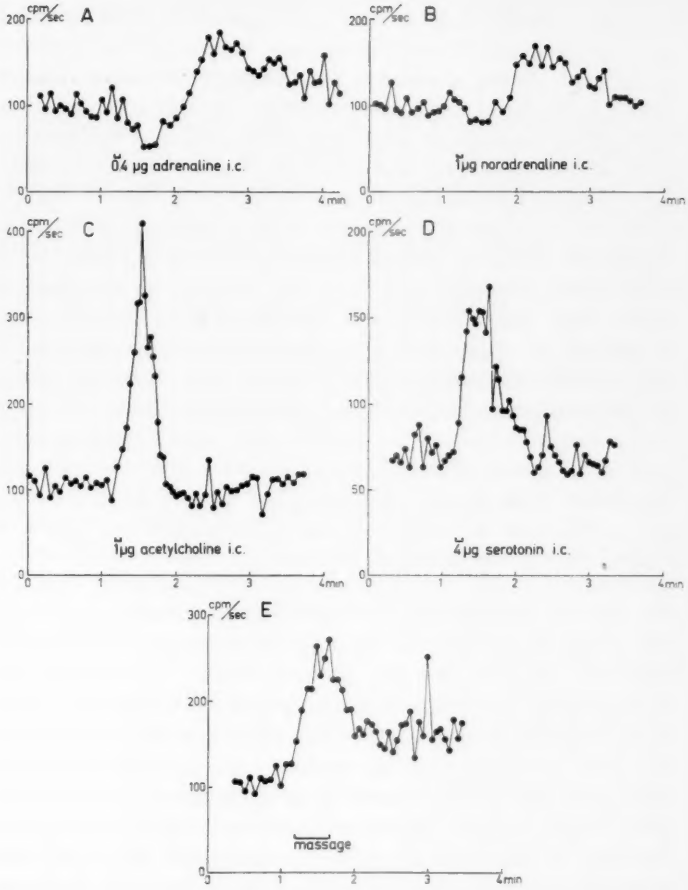

Fig. 59. Same cat as in Fig. 58. The figure shows the corresponding graphs of the rate of release of labelled thyroid hormones. Testing procedures indicated on the records. Full description in the text (i. c. = injection into the common carotid artery, "massage" = mechanical stimulation of the gland with forceps). *Note:* Calibration in *D* differs from that of *A* to *C* and *E*.

There seems to be some relation between the depressed rate of secretion and the circulatory response although further study is required to reveal the details. The exhaustion may, for example, be dependent on activities elicited to counteract the systemic circulatory response caused by the hormone. The acetylcholine was also administered by direct arterial injection to reduce the systemic effect of the hormone. Such an experiment is reproduced in Figs. 58 and 59 from a cat under light Nembutal anaesthesia whose laryngeal nerve was cut. Fig. 58 shows the circulatory responses, Fig. 59 the corresponding effects on the rate of release of labelled hormones. *A* in both figures is the effect of 0.4 micrograms of adrenaline injected into the common carotid artery below the thyroidal branches. The carotid artery was ligated one cm above the main laryngeal artery to prevent acetylcholine from reaching the head. *B* shows the effect of one microgram of noradrenaline, *C* that of one microgram of acetylcholine, *D* that of four micrograms serotonin (*cf.* below), all administered by the same route as the adrenaline in *A*. Finally *E* shows the result of mechanical stimulation of the gland with forceps. The effect of all these stimuli was a substantial increase of the rate of secretion of hormone with little or no sign of exhaustion afterwards.

Serotonin

Hydroxytryptamine also favoured the discharge of hormonal radioiodine from the thyroid treated with TSH. The effect was weaker than that of acetylcholine but probably more marked than that of adrenaline. The serotonin can only be administered by the arterial route; otherwise rapid break-down occurs, especially in the lungs. Intravenous administration also elicited severe changes in systemic circulation frequently resulting in 'arousal reactions.' Fig. 59 *D* shows the effect of four micrograms of serotonin injected into the left common carotid of the same cat as that used for the demonstration of the effect of catechols and acetylcholine. For comparison the thyroid was then massaged to produce a mechanical discharge of hormone *(E)* as demonstrated by HEKTOEN, CARLSON and SCHULHOF (1927). These authors used a precipitin reaction to assay thyroglobulin and measured

the tyroid discharge of thyroglobulin into the blood and lymph of goiterous dogs.

Antidiuretic hormone

Intravenously administered Pitressin had no effect on thyroid hormone secretion unless the vasoconstriction elicited was very pronounced and long-lasting. In such cases there was sometimes a slight reduction of the rate of release of hormonal iodine, but the secretory activity rapidly recovered when vasoconstriction subsided.

Circulatory failure and oxygen lack

Very little occurred in the thyroid in response to oxygen lack or circulatory failure and thyroid hormone secretion could in fact be demonstrated even after death by perfusing the thyroid with unoxygenated blood from the same animal.

Anaesthetics

The thyroid mechanism for hormonal release was little influenced in acute experiments by the anaesthetics used. The only significant result was a small transient reduction after the rapid injection of chloralose. This general observation is in conformity with the remarkable insensitivity of thyroid secretory functions to oxygen lack and circulatory failure. Consequently, thyroid hormone secretion certainly occurred even when the synthesis of fresh hormone was inhibited by the anaesthesia (*cf.* p. 92).

The pituitary release of TSH in relation to anaesthetics has not been studied in detail since the effect of the operation could not be excluded. However, as was described in the beginning of this chapter, signs of endogenous TSH activity could appear late in the experiment although the level of anaesthesia was generally kept as constant as possible and sometimes deep enough to give a relatively insensitive electroencephalogram with 'spindle bursts' and slow waves. In one cat under very light Nembutal anaesthesia which permitted marked shivering that could be potentiated by adrenaline, the initial signs of hormone release soon disappeared. Conversely, in a second cat under anaesthesia of sufficient

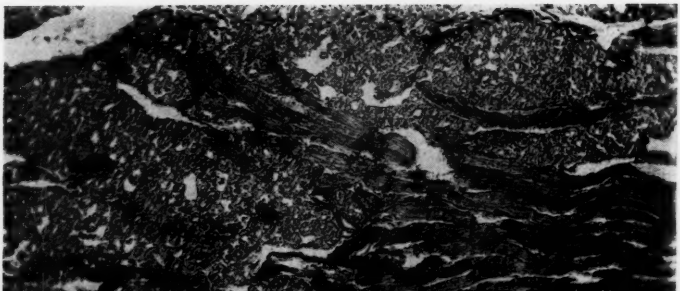

Fig. 60. Paraffin section of thyroid gland of a rabbit stained with Heidenhain's azan. The gland was large and active. *Note:* Muscle bundles following interlobular septa of connective tissue.

depth to abolish shivering and the 'arousal' response to noiceceptive stimuli, a marked increase in the rate of release of hormonal iodine was observed.

Musculus levator glandulae thyreoideae

Muscle bundles ending in the thyroid capsule have been found in two of the animals studied histologically (Fig. 60). They are probably aberrant fibres from the thyrohyoid, cricothyroid or laryngopharyngical muscles (EISLER, 1900), and are reported not to occur regularly (KOPSCH, 1939). It is not likely that they can exert any measurable effect on the thyroid gland. Sufficient mechanical stimulation to release any iodine compounds including thyroglobulin was in fact found to require such force that tissue damage could explain the effect. Such an experiment in which the thyroid gland was pinched with forceps is shown in Figs. 58 and 59 *E* on p. 74–75.

The relation between uptake of radioiodine and rate of secretion of labelled hormone

As has already been reported in previous chapters, I^{132} was tested against I^{131} in uptake experiments and these two isotopes were found to behave identically. The isotope with the short half-life (I^{132} 2.3 hours) was then used to measure the rate of uptake of iodine in experiments on animals in which the hormone had been labelled with I^{131} by the administration of the tracer one to two days before the acute experiment (see previous chapter). The results obtained were in good agreement with the assumptions made by comparing experiments measuring the rate of uptake with those measuring the rate of secretion.

The technique was also used in animals pretreated with TSH to study how the increased uptake of iodine after adrenaline was related to the increased secretion of labelled hormone. From measurements on different animals, rapid changes in these two variables (uptake of iodine and hormone secretion) could not easily be compared, because they do not appear in exactly the same time relation to the stimulus. An experiment with two isotopes is shown in Fig. 61. The upper part of the figure is a reproduction of the original record of blood flow and systemic blood pressure. Below these, two horizontal lines show the relation between the time scales of *A* and *B*. The bottom record B, is a graphical reproduction of rate of release of I^{131} which was bound to the thyroidal hormone (filled circles) and the rate of uptake of inorganic I^{132} that was administered at the beginning of the experiment. Since neither of the two types of measurement is quantitative the two curves are not quantitatively comparable. The scales of concentration of the isotopes are arranged to facilitate a comparison of the shape of the curves, both scales being given in counts per minute per second *i. e.* the release or uptake of radioactivity per unit time obtained by multiplying the final values of their corresponding V–A or A–V differences by the value of the rate of flow. Since the arterial and venous concentrations of I^{132} are obtained by differences of values of concentrations of I^{131} measured at

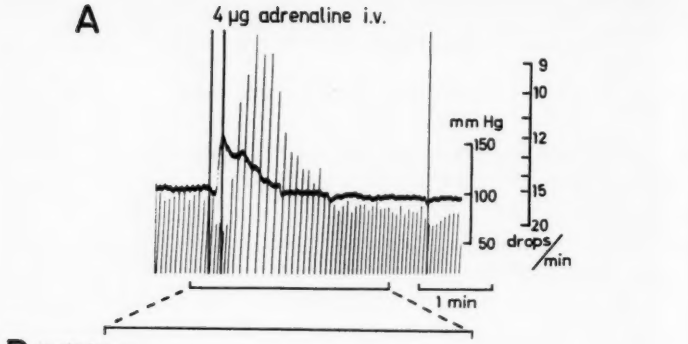

Fig. 61. Rabbit. 2.5 kg. Nembutal-chloralose. Thyroid hormones labelled with I^{131}. The animal was not pretreated with NaI^{127} but the fraction of I^{131} as iodide still circulating in the blood was found negligeable. Secretion of thyroid hormone was induced by a large dose of exogenous TSH. *A*, blood pressure from the femoral artery (heavy horizontal line) and time intervals between blood drops from a cannula in the thyroid vein (steeply rising lines). *B*, rate of uptake of I^{132} (open circles) and rate of release of hormone labelled with I^{131} (filled circles). Calibration on the left in counts per minute per second (cpm/sec) *i. e.* radioactivity taken up or released per unit time. Time relation between *A* and *B* is illustrated by horizontal bars between the figures. Vertical broken line in *B* indicates the onset of the injection of 4 μg adrenaline. The adrenaline constricted thyroid vessels with short latency and increased rate of release of hormonal I^{131} with a latency somewhat less than one minute. Rate of uptake of I^{132} was reduced initially but then increased above the original level.

a time when practically all the I^{132} had disappeared, the errors are even larger than usual. The main effects are, however, seen in the curves. Between the two vertical lines in *A*, 4 micrograms of adrenaline were injected intravenously. The onset of this injection is marked by a dashed vertical line in *B*. The usual effects of adrenaline are seen: i) the thyroid vasoconstriction, ii) the increased rate of secretion of labelled hormone which appears with a latency of about 40 sec, iii) the reduced rate of uptake or radioiodine that always occurs during vasoconstrictions and iv) the increased rate of uptake that is seen after adrenaline in animals pretreated with TSH.

The increased rate of hormonal secretion did not correlate in time with either the period of depressed uptake or with the period of increased uptake, the latter appearing somewhat later than the effect on the release. It should be stressed that these effects are not intimately related. The period of increased uptake can be obtained a few minutes after the intravenous administration of TSH before there are any signs of hormonal release. As was also stated above, protein binding of accumulated iodine does not necessarily occur in experiments with a high rate of secretion. Yet, if this be the case, hormone made of freshly accumulated iodine is not labelled to the same extent as that made of previously accumulated ions and is therefore not detected by the tracer technique. At the end of the multitracer experiments large doses of NaI^{127} were administered so that the u p t a k e of I^{131} could be determined.

Uptake of labelled phosphate by the gland

Only two experiments were performed to study the rate of uptake of phosphate since it had been calculated from the work by BORELL, 1945; BORELL and HOLMGREN, 1949; LAMBERG, 1953; MASON, 1953 and others that the A–V difference of P^{32} would be small in comparison with the arterial concentration. It should also be remembered that the total blood phosphorous is 5 mg per cent or about 1 000 times the iodine concentration. It was also noted in both rabbits studied that the A–V difference was insignificant in relation to the arterial concentration. Further, the rate of change of the arterial concentration made it difficult to obtain accurate values. Control tests with I^{131} showed that the uptake of iodine by the gland was normal.

Other studies of the general metabolic rate of the thyroid were not performed but successful results would probably be obtained with measurements of the oxygen consumption, since even direct observation of the thyroid venous blood of the rabbit showed that its colour sometimes varied regardless of rate of flow and arterial oxygen saturation. This seemed to be the case particularly after large doses of TSH.

DISCUSSION

Sources of error

The validity of the described results obviously depends on how well the experimental technique measures first, total thyroid venous outflow and second, venous radioactive iodine concentration . Errors in measurements of venous outflow may be related to the following possible factors:

i) the presence of anastomotic communications,
ii) net fluid shifts from blood to thyroid tissue and *vice versa,*
iii) variation in drop volume,
iv) anaesthesia and operative interference,
v) inadequate maintenance of circulating blood volume,
vi) heparinization.

Errors in the assay of radioiodine isotopes may arise from the following:

i) sedimentation of blood cells in the cannula,
ii) heterogenous distribution of isotopes in labelled substances,
iii) isotopic labelling of organic compounds other than thyroid hormones,
iv) radiation damage,
v) the process of radioactive counting.

Reference to several of these factors has already been made in the description of the experimental method. Further discussion of sources of error and the origin of artefacts follows below.

Anastomoses

It has been possible to determine by direct inspection that most vascular anastamoses to extra-thyroidal regions are interrupted by the operative procedure. However, those anastomoses which escape dissection may not be insignificant and they may be classified into two varieties: i) vessels conducting blood that has perfused tissues other than thyroid before it flows through the cannula, ii) vessels that conduct part of the thyroid venous blood back to the general circulation. Anastomoses of the first type may influence rate of flow and A–V difference, but in opposite directions. Therefore, measured rate of uptake and rate of secretion will not be disturbed.

Anastomoses of the second type, however, will reduce the amount of thyroid tissue that is studied and thereby depress the values obtained for both the rate of uptake and the rate of secretion. Vasomotor reactions may change the proportions of venous drainage through the cannula and these anastomoses. Furthermore, these veins would permit labelled hormone to enter the general circulation. Anastomoses of this type must be relatively large and should therefore always be seen by the naked eye. A simple manoeuvre will also demonstrate their presence. When the free end of the cannula is raised, blood flow stops or is reversed if these vessels are present; if they are absent, venous pressure will increase and thyroid oedema should develop. An important property of anastomoses of the second type is that they will affect the measured values of rate of uptake and rate of secretion equally and simultaneously. However, equal and simultaneous changes in these two indices were never observed. Although this is not conclusive evidence it suggests that the influence of such anastomoses is small.

The presence of arteriovenous shunts within the thyroid gland may also confuse the interpretation of results. Such shunts have been reported by HORNE (1892) and MODELL (1933) and they were observed during the present study after the injection of a few gas bubbles into the carotid artery. The bubbles rapidly penetrated the thyroid and appeared soon after the injection in the vein and the cannula. Modell assumed that the arteriovenous communications regulated the blood flow through the active parts of the thyroid. This assumption

suggests that increased rate of flow accompanies decreased A–V difference of radioiodine, but this was not observed in the rabbits and but rarely, to a small degree, in cats. On the contrary, the A–V difference regularly increased with increasing rate of blood flow. Therefore the effect of arteriovenous communications inside the thyroid gland is probably small. Measurement of the total uptake of iodine over a long period of time both with direct determination of clearance and with assay of the content of radioiodine in the whole gland should make it possible to clarify this question.

In conclusion, it is believed that blood flow as recorded is representative of the total thyroid blood flow. Contamination with extrathyroidal blood can account for only small and insignificant distortions of the curves of A–V differences.

Net fluid shifts

Diffusion of fluid from the blood to thyroid tissue (oedema) or *vice versa* should, in the following types of experiments, cause transient changes in the haematocrit of thyroid venous blood and so yield A–V differences other than zero: i) when thyroid uptake is inhibited by a drug or by prolonged suppression of TSH secretion, ii) when apparent thyroid uptake is inhibited by large doses of NaI^{127}, iii) when thyroid hormone secretion is inhibited by lack of TSH. However, the A–V difference in these experiments was always zero. This proved that rapid changes in haematocrit did not occur.

Changes in drop volume

This question has been discussed by LINDGREN (1955) who calibrated the rate of flow in volume per time from measurements of the drop volume at different drop rates. The fact that A–V difference was zero in experiments in which the net uptake or release of iodine was zero also proves that the changes in drop volume are negligeable in the present experiments. However, slow variations in drop volume did occur over a long period of time when changes occurred in the haematocrit of the blood of the general extracapillary circulation but such variations did not affect observations on A–V difference.

Anaesthesia and operation

These factors cannot be avoided. It was however found that the anaesthetics employed had little immediate influence on the thyroid gland except for the inhibition of the organic binding of iodine. This was sometimes considerable.

Maintenance of circulatory blood volume

This question will be discussed below in relation to baroceptive influence. Extracorporeal circulation of blood may lead to liberation of depressor substances (*cf.* GRUNDY and HOWARTH, 1957) which may alter thyroid function. Changes in haematocrit may be produced (*cf.* above).

Heparinization

All experiments were performed on heparinized animals. Heparin has been reported to inhibit or reduce some responses to stressing stimuli in the rat (HAMILTON and LOWENTHAL, 1956). Similar effects may possibly influence hormonal activity in the rabbit and cat. Heparin administration also leads to a marked increase in unesterified fatty acids in the plasma (GROSSMAN, MOELLER and PALM, 1955; GROSSMAN, PALM, BECKER and MOELLER, 1954 and SPITZER and MILLER, 1956).

Sedimentation of blood cells in the cannula

Theoretically, sedimentation may cause a large artefact since 9 times as much protein bound iodine is found in the plasma as in an equal weight of red blood cells (COURRIER, 1957), and the cellular iodide of the blood is only 27 per cent of the iodide of the whole blood (RIGGS, LAVIETES and MAN, 1942; COURRIER, 1957). The blood volume of the anaesthetized rabbit is about 50 ml per kg body weight, the corresponding value for the cell volume being 20 ml (MCKINNA and MISIEWICS, 1954). However, it is reported above that the A–V difference in the thyroid was zero regardless of flow rate when the glandular functions were inhibited. This suggests that sedimentation artefacts are negligeable.

Distribution of isotopes

The use of a record of the release of labelled hormone as a measure of true hormone secretion demands that the secreted hormone be uniformly labelled. It is generally agreed that this is the case at least to the extent that estimations of release curves can be made from direct measurement of the total radioactivity of the thyroid. However, the present method is sensitive to degrees of change in the gland content of radioactivity that have never been studied before. Many of the effects described here are of an order of magnitude that would not significantly influence average values measured over a period of a few hours. Evidence has been presented by LUDFORD and CRAMER (1928) that the thyroid may have two ways of secreting its hormones. One is transport of preformed hormones from the colloid through the cells to the blood stream whereas the other is a direct release of freshly synthetized hormone directly from the cells to the blood. There is no support in the present study for differential action of this kind. Since in the present experiments there is no uptake of radioiodine of the isotope that has labelled the hormone, hormone synthetized during the experiment could not contain any radioactive isotope of the kind used for labelling the general store. If direct mechanisms of the postulated kind existed, a switch from secretion of a small amount of labelled hormone to a large amount of unlabelled hormone, caused by stimulation with TSH, should either cause a decrease in the rate of secretion of labelled hormone or not influence it at all. In the author's experiments the release after TSH has been of an impressive magnitude. Possibly, the validity of the above-mentioned hypothesis could be tested by a multi-tracer technique making use of two isotopes with long half-lives.

No distinction has been made in this work between the various thyroid hormones that have been reported to exist (GROSS and PITT-RIVERS, 1952, 1953; ROCHE, LISSITZKY and MICHEL, 1952; TAUROG, WHEAT and CHAIKOFF, 1955; ACKERMAN and ARONS, 1956) since compounds which have less iodine per molecule of hormone than thyroxine are assumed to have negligeable influence on the rate of release of protein bound iodine. However, the physiological actions of these compounds may differ from thyroxine both in latency and mode of action.

Radiation damage

To improve the sensitivity of the metod, rather large doses of I^{131} had to be given. The possibility of radiation damage must therefore be considered. Traces of thyroglobulin in the venous blood which appear at an early stage in radiation damage were not detected in the present experiments. TAUROG *et al.* (1955), using 10 times larger doses of radioiodine, did find signs of injury. However, even the smaller dose of tracer in our experiments is very near the threshold for eliciting radiation damage. The results were largely the same whether the radioiodine had been administered one or three days before the measurement. Now, since the effect of radioactivity is related to the exposure time, this observation also indicates that radiation damage was not severe. Histological controls of important experiments also showed normal structure (Fig. 62). (For further discussion of radiation damage, see HAMILTON and LAWRENCE (1942), MYANT (1953) for the rabbit and LARSSON (1955) for a general review.)

Baroreceptor and 'volume receptor' influence

The results of blood flow measurements confirm earlier findings by REIN, LIEBERMEISTER and SCHNEIDER (1932) that the vasomotor tone in the thyroid is intimately related to baroreceptive activity. The high sensitivity of thyroid vessels to intravenous infusions as well as to electrical stimulation of the central end of the cut vagus suggests that the influence of pressure receptors is not restricted to the action of those in arterial walls. Intravenous injections of a small amount of fluid have in fact more effect on the pressure of the large veins and the right atrium where the well known blood 'volume receptors' are situated (HENRY, GAUER and REEVES, 1956) than on the arterial blood pressure influencing for example the carotid sinus. This has to be remembered when evaluating the results of pharmacological studies of the thyroid both with and without its nerve supply. In either case blood vessels are sensitive to the physiological doses of ADH and catechols which may be released in response to increased venous pressure. This, in particular, has compli-

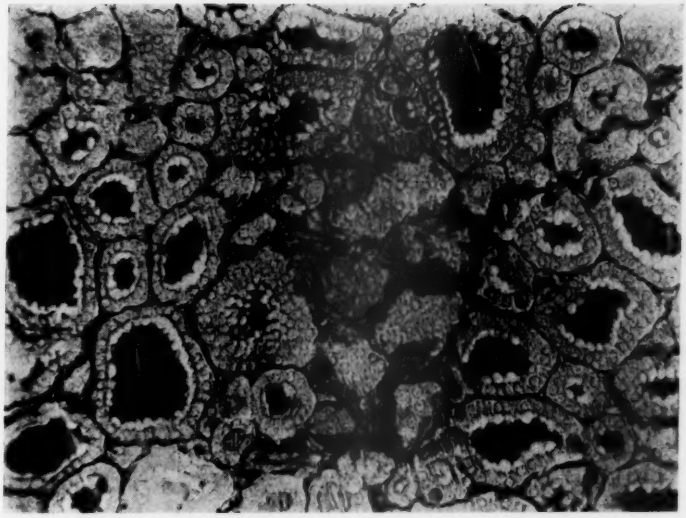

Fig. 62. Paraffin section, stained in Heidenhain's azan, of a normal rabbit thyroid taken from the animal after an experiment in which rate of release of labelled hormones was measured. Secretion induced by exogenous TSH. *Note:* Large number of colloid droplets on the interior walls of the follicles. Black spots between follicles are red blood cells in the capillary network around the follicles.

cated the present experiments because the venous outflow from the gland, which at its best amounted to $^1/_{10}$ of the total volume in one minute, had to be carefully compensated for by intravenous re-infusion. Long term effects on the blood flow from thyrotropic hormone, for example, have therefore to be evaluated with care. The 'volume receptor' reflex may also influence the vasodilatatory effects observed in 'arousals,' not only indirectly owing to redistribution of blood within the body but also directly, by central nervous changes of reflex sensitivity. This question probably deserves further experimental studies in which the thyroid may be used as a sensitive index. The vasoconstriction sometimes observed in the gland during local heating of hypothalamus is perhaps another example of an effect closely related to pressure receptors. Similarly preliminary experi-

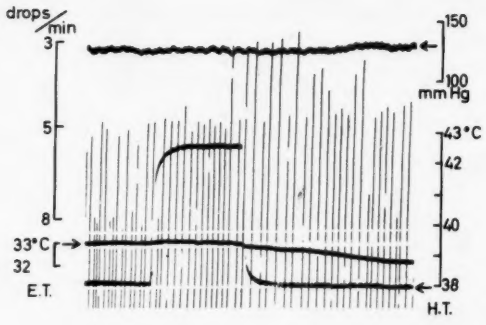

Fig. 63. Cat. 4.0 kg. Nembutal. Heating electrodes in anterior hypothalamus. *Continuous curves from above:* Femoral blood pressure (calibration on the right), ear skin temperature, *E. T.*, (calibration on the left) and hypothalamic temperature, (calibration on the right). *Steeply rising lines:* Time intervals between successive drops of urine from the free end of a cannula in the left ureter. 'Water diuresis' induced by intravenous infusion of 5 % glucose. Note, transient reduction in urine flow when hypothalamus was heated from 38° to 42.5° C.

ments using urine flow as an index have shown that water diuresis may be rapidly inhibited by hypothalamic heating as demonstrated in Fig. 63 where this effect had a still lower threshold than the inhibition of the cutaneous vasomotor tone, measured as increased ear skin temperature. (Changes in water balance in response to changes in body temperature were demonstrated by BARBOUR; for references see BARBOUR, 1940).

Uptake of iodine

The uptake of iodine from the blood is mainly dependent on two processes in the thyroid, i) diffusion of iodide until equilibrium is established between blood and the iodide space in the gland, ii) rate of organic binding of trapped iodide (RIGGS, 1952). The first process is a real diffusion which can go in both directions but is dependent on some mechanism in the gland that can maintain a high concentration gradient towards the blood (*cf.* RIGGS, 1952). The diffusion

of iodide is probably as rapid as diffusion of other ions in most tissues (PAPPENHEIMER, 1953) since the A–V difference only rarely declined when the thyroid flow increased. Rate of flow is therefore probably not the factor limiting the rate of diffusion. It can even be calculated from the distribution of iodide between red blood cells and plasma, described above, that some of the cellular iodide is also available to the thyroid when blood passes through the capillaries.

The rate of organic utilization of iodine influences the uptake by removing iodine from the iodide space, thus permitting more iodide to diffuse into the space from the blood. Of all the reactions studied in the thyroid this one seems to be most sensitive to anaesthetics. This will be discussed below in relation to the measurement of clearance.

One manner in which the diffusion of iodide to or from the thyroid may be varied is a change in the iodide space of the thyroid. If one assumes that the concentration gradient of iodide between the inside and the outside of cells and follicles is maintained by a loose binding of iodine or by a Donnan equilibrium, it is reasonable to conclude that such a change of the iodide space may readily occur.

Iodine clearance

BROWN-GRANT, C. VON EULER, HARRIS and REICHLIN (1954) found that clearance values began to decrease 30 to 60 min after the administration of I^{131}. They concluded that the effect was caused by thyroid hormone which had been synthetized after the administration of the tracer and had therefore been labelled with I^{131}. The finding of decreased clearance values in the present experiments can hardly be explained in the same way since the rate of synthesis of hormone was apparently reduced by the anaesthesia and the condition of stress. Furthermore, the clearance declined somewhat more rapidly in animals not treated with exogenous TSH than in those which were treated and so had a high rate of hormone release. The decline of the clearance is not fully understood. It may be related to the inhibition of organic binding of iodine by anaesthetics or to an unknown reduction in the iodide space of the thyroid.

It is evident that the rate of uptake of iodine by the thyroid can be

equated to the rate of hormone secretion under conditions of iodine equilibrium. This is the theoretical basis of uptake measurement as an index of thyroid activity. It certainly holds true if the periods of uptake measurement are long enough. BROWN-GRANT and GIBSON (1955) have beautifully demonstrated that 24 hours are not enough because exogenous thyroxine in a dose that within this period completely inhibited hormone secretion, reduced the rate of uptake only by about 50 per cent. If the uptake is studied for the short periods necessitated by present experiments it will be of prime importance to know the size of the iodide space of the thyroid. BROWN-GRANT and GIBSON (1955) found that the utilization of accumulated iodine for the synthesis of organic compounds was rapid and complete in control rabbits, whereas the iodide trapped by the glands of those treated with 50 to 100 mg methylthiouracil could be completely discharged by administration of potassium thiocyanate. The author's results in anaesthetized animals without thiouracil lie between their values in thiouracil treated and control rabbits, since part of the trapped radioiodide could diffuse from the gland to the blood after the injection of NaSCN or large doses of NaI^{127}. In Brown-Grant's experiment the uptake of iodine of thyroids treated with thiouracil was almost complete after 65 min when equilibrium between blood and thyroid had been established. In the present experiments significant uptake was measured as late as 7 hours after the injection of the tracer. The rate of uptake responded in a similar way to various stimuli regardless of whether rate of uptake was studied early in the experiments or more than 65 min after the administration of radioactive iodine. However, the interpretation is complicated by the fact that inhibition of organic binding of trapped iodine is probably of shorter duration after barbiturates than after thiouracil. It should be noted that thiopental (which has not been extensively studied by the author) according to WASE and FOSTER (1956) has an effect of up to 200 hours duration.

Hormone secretion

The method has revealed that intravenously injected TSH can induce a measurable hormone secretion within 5 to 10 minutes after its administration. The half-life of the effect of TSH was one to four

hours in the rabbit, in good agreement with the result of REICHLIN and REID (1955). It was longer in the cat but not regularly measured.

The fundamental finding that nervous and hormonal stimuli other than TSH had no effect in the absence of the latter but could markedly influence a secretion induced by TSH, should be emphasized. It shows that there are no true secretory nerves to the thyroid gland but that various stimuli can affect the sensitivity of its cells to TSH. Nervous influence, on the other hand, is not necessary for thyroid function since transplants of thyroid tissue secrete hormone in response to TSH (MANLEY and MARINE, 1915; MANLEY, 1916). Also significant is the finding that electrical stimulation of the cervical sympathetic trunk or the superior laryngeal nerve elicited a thyroid response which was often smaller and flatter than the response elicited by reflex stimulation. It suggests that the sympathetic action on the thyroid may sometimes be inhibitory which is in agreement with the observation that large doses of adrenaline and noradrenaline transiently inhibited thyroid activity. It is also tempting to draw a parallel between the thyroid and the sympathetic ganglia, for in the latter transmission is blocked by physiological concentrations of adrenaline (LUNDBERG, 1952). However, inhibition in the thyroid could not be obtained with serotonin even in a dose of 4 micrograms injected into the artery, although serotonin also had been reported to inhibit the transmission in ganglia (MARAZZI and HART, 1953). Another explanation of the inhibitory effect of adrenaline is that the hormone depresses the rate of metabolism of the glandular cells. Such an inhibition has been observed in other tissues both *in vitro* and *in vivo* (*e. g.* U. S. VON EULER, 1954).

Cells in the salivary glands which are innervated both by sympathetic and parasympathetic fibres have been reported to respond with secretion to stimuli from either of these nerves and the two kinds of fibres have, furthermore, synergic action (for references see LUNDBERG, 1958). Similar properties are probably not found in the thyroid where it seems necessary to stimulate special nerve fibres in order to obtain increased hormone secretion. Whether or not these nerve fibres are secretory fibres in the sense that they innervate follicle cells alone, is not known. NONIDEZ, 1931–1935 for anatomy and

histology of thyroid nerves). One microgram of acetylcholine injected intravenously is sufficient to facilitate the hormonal release. As stated on p. 61 even if fibres had been found to innervate glandular cells selectively, they can never be called true secretory fibres because they act only by changing the sensitivity of the thyroid to its governor in the pituitary. At zero level of secretion they have been shown to be without effect.

Serotonin

The action of serotonin on glands has not received much attention in the literature. This is remarkable in view of the wide-spread occurrence of enterochromaffine cells, the high concentration of serotonin in several organs, and the variety of autonomous effects of the hormone which have already been studied (see ERSPARMER, 1954; PAGE, 1954; UDENFRIEND, SHORE, BOGDANSKI, WEISSBACH and BRODIE, 1957). The only result comparable to our observation that serotonin increases the rate of thyroid hormone secretion is a report by REID and RAND (1951) that serotonin releases catechols from the adrenal medulla. It may also be mentioned that BACQ and GHIRETTI (1951) and BACQ (1952) have reported that electrical stimulation of the secretory nerve of an isolated salivary gland of *Octopus vulgaris* and of *Eledone moschata* releases serotonin both into the perfusate and into the saliva. It is known that blood thrombocytes have a high concentration of serotonin. This may influence experiments in which damage to these corpuscles can occur, such as perfusions and injections of foreign substance into arteries close to the organs.

Seasonal variations

The observation that thyroid activity of the rabbits decreased during the spring and summer may be a sign of adaptation to the increased environmental temperature. Animals kept in a cold room for a few days also had somewhat larger glands than other rabbits and probably higher rates of blood flow although no attempt was made to verify this statistically. Thyroid activity may also change in response to the diet inasmuch as it may contain goitrogens. CHESNEY,

CLAWSON and WEBSTER (1928) showed that rabbits became goiterous when they were fed on diets containing cabbage. The animals used in this work were not kept on standard diets prior to the day they were delivered to the laboratory (generally 3 to 15 days before the experiment). Also, the content of goitrogens in plants shows seasonal variations and is low in the spring and summer (SPENCER, 1934). For further references to seasonal variations see SEIDELL and FENGER (1913), RING (1939), UOTILA (1940), MEANS (1948) and PICHOTKA (1953).

The thyroid-pituitary relationship

The 'feed-back' or 'servo-mechanism' control of thyroid activity, recently reviewed by BROWN-GRANT (1957) implies that thyroid hormones inhibit the pituitary secretion of the thyroid stimulating hormone (TSH) so that some equilibrium between thyroid and pituitary activities is established. This notion has developed from a large number of observations (BROWN-GRANT, 1957, for references). The 'receptor,' sensitive to thyroid hormones, is located in the anterior pituitary itself since transplants in the anterior chamber of the eye still respond with inhibition of the TSH secretion when thyroid hormone concentration of the blood is increased (C. VON EULER and HOLMGREN 1956 *a*).

The pituitary-thyroid activity equilibrium can be shifted in any direction and thus thyroid activity may be adjusted to the needs of the body. This 'setting of feed-back' is impaired in animals in which the pituitary is removed and replaced by a transplant in the eye although the transplant itself still responds to increased thyroid hormone concentration (C. VON EULER and HOLMGREN, 1956 *b*).

The mechanism regulating the equilibrium between the pituitary and thyroid activities is still largely unknown. BOTTARI (1957) has recently presented strong evidence to the effect that the pituitary does not react rapidly to changes in the blood concentration of thyroid hormones. The pituitary, therefore, might respond not to the hormonal blood level but rather to the amount accumulated in the hypophysis. Rate of uptake and rate of breakdown of *e. g.* thyroxine within the pituitary might then determine TSH output.

The blood thyroid hormone concentration may be dependent not only on the thyroid output but also on the rate of diffusion of its hormones into various tissues. This rate of diffusion is probably related to the amount of free thyroid hormones in the blood. However, most of these hormones are bound to a protein, 'the thyroxine-binding protein,' one third of which is normally utilized by thyroid hormones (ALBRIGHT, LARSON and DEISS, 1955). There should consequently be an equilibrium between free and bound hormones in the blood determining rate of diffusion into tissues, including the pituitary. (For a discussion of changes in the thyroxine-binding protein, see PETERS, MAN and HEINEMANN (1948), DANOWSKI, JOHNSTON and GREENMAN (1950), HORST and RÖSLER (1953), DOWLING, FREINKEL and INGBAR (1956) and ROBBINS and RALL (1957).)

The efferent effect of the pituitary in this mechanism is the secretion of TSH; when this hormone is taken up by the thyroid, it exerts an influence there (RAWSON, STERNE and AUB, 1941; SONENBERG and MONEY, 1955). TSH is also taken up and inactivated by other tissues. LEVEY and SOLOMON (1957) found that only one third of a dose of TSH could be detected in the plasma after 5 min and only a few per cent after one hour. The proportion that reaches the thyroid may therefore be influenced both by the rate of destruction in extrathyroidal tissues (GYLLENSTEN, 1953; D'ANGELO, 1955) and by the rate of thyroid blood flow. This mechanism obviously cannot influence the level of activity to which the thyroid is adjusted by the 'feed-back,' but it might cause oscillations in that activity. A 'feed-back' means that the TSH level of the blood is not a good index of thyroid function, and it is also a well known fact that blood TSH reaches its highest values in myxoedema and is generally low but highly variable in thyrotoxic patients (HERTZ and OASTLER, 1936; COPE, 1938; GALLI-MAININI, 1942; PURVES and GRIESBACH, 1949; D'ANGELO and GORDON, 1950; D'ANGELO, PASCHKIS, GORDON and CANTAROW (1951) and SIMKIN, STARR and HANCOCK (1952).

With a new and remarkably sensitive method of assay of thyrotropin, BOTTARI (1957) has demonstrated that the blood TSH sometimes can give valuable information about rapid changes in the pituitary-thyroid relationship. Thus, exposure of rabbits to a moder-

ately cold environment caused an increase in the blood concentration of TSH measurable with Bottari's method after 30 min, with a peak value after three hours when a 500-fold increase could be noted. Injection of thyroxine immediately before the exposure to cold did not significantly inhibit this effect but it was greatly reduced when the thyroxine had been injected three hours before the exposure. These observations indicate that the pituitary response to changes in the blood thyroxine is slow while the reaction to cold is rapid. They also show that increased pituitary activity in response to cold is not necessarily due to increased peripheral break-down of thyroxine.

The thyroid pituitary system has been extensively discussed because the rate of change of a few of its variables may influence the system as a whole. The present study has been made with a technique that involves the interruption of the 'feed-back' loop proposed in the literature, because the secreted thyroid hormone is not available to the animal. The responsiveness of the thyroid can therefore be described in traditional terms of latency and duration of the effects elicited in the experiments. The following temporal characteristics have been measured:

i) The thyroid response to TSH is rapid. Significant effects can be measured after 5 to 10 min. However, the effect of TSH in reduced and its onset is retarded by vasoconstriction in the thyroid.

ii) The half-life of the TSH effect is a few hours in the rabbit. This verifies the measurement of REICHLIN and REID (1955). It is probably even longer in the cat. The thyroid sensitivity to a given input of TSH varies rapidly and profoundly with changes in nervous and hormonal influence.

Several other temporal characteristics of the thyroid pituitary system have already been estimated, for example, the half-life of the pituitary response to thyroid hormones seems to be as long as 20 to 30 hours (see *e. g.* BROWN-GRANT, 1957) and the onset of the pituitary response to increased thyroxine a few hours (*cf.* BOTTARI, discussed above). Further experimentation has to be done to measure these time courses with certainty under various conditions.

In addition, it would be desirable to study the importance of the equilibrium between free and protein-bound thyroid hormones in the blood, especially since it has been demonstrated in this work that

thyroid hormones are not necessarily secreted at constant rates but that the rate of release may vary from minute to minute.

A consequence of the possibility of reduced uptake of TSH by the vasoconstricted thyroid is that, owing to the 'feed-back,' the TSH level of the blood may rise in response to a prolonged vasoconstriction. Whether or not this is the explanation of the finding by BOTTARI (1957) and BOTTARI and MARTINI (1958) that antidiuretic hormone causes an increase in the TSH level, has to be examined. The dose of Pitressin given in their experiments (0.2 units per kg body weight) was probably high enough to cause considerable thyroid vasoconstriction.

Iodine shortage

The present experiments have confirmed earlier findings that the latency for inhibiting the release of thyroid hormone is shorter than that for inhibiting uptake of iodine as far as these effects are mediated by the anterior pituitary. However, there is also a short term reaction in the thyroid which can reduce uptake of iodine by vasoconstriction. The secretion of hormone, on the contrary, does not seem to be severely influenced by vasoconstriction. This would imply that there may be situations of reduced uptake of inorganic iodine with uninfluenced release of hormonal iodine (*cf.* SHIPLEY and MACINTYRE, 1954). This is probably of little importance in the normal euthyroid man and animal since the daily iodine consumption of the thyroid is only about one μg/kg body weight, less than one per cent of the total iodine in the thyroid (*cf.* RIGGS, 1952 and other monographs). From calculations of the normal blood level of iodine in man, one would assume that there is a good safety margin permitting considerable periods of vasoconstriction. This is also supported by the finding of POCHIN (1950) that the extraction efficiency of man was as low as 0.2. However, at times of reduced intake of iodine, repeated vasoconstrictions, caused by stress for example, may easily reduce the uptake of iodine by the thyroid below the critical level determined by the output of iodine in the thyroid hormones. At the same time the stress situation may also increase the loss of iodine in the urine. It has been suggested that the hormone production be-

comes difficult when the total iodine concentration in the gland has fallen below 50 per cent of the normal value (LEVINE, REMINGTON and VON KOLNITZ, 1933). CHILDS, KEATING, RALL, WILLIAMS and POWER (1950) came to a similar conclusion for they showed that the biosynthesis of thyroxine may be depressed when the blood level of iodine falls below 5 micrograms per 100 ml blood. From these figures the importance of increased vascularization of the thyroid in iodine-deficiency becomes apparent. The efficiency of the mechanism for filtering off the iodine in the blood is in fact so high in patients with iodine-deficient glands that they may remain in positive iodine balance for weeks after a quantity of iodine equivalent to that ingested by inhabitants of nongoitrous areas has been added to the daily diet (STANBURY, BROWNELL, RIGGS, PERINETTI, DEL CASTILLO and ITOIZ, 1952).

Thyroid storm

Since thyroid activity may be influenced even in moderate anaesthesia both by nervous and hormonal activity, experimental evidence is provided for a mechanism that might explain the thyroid crisis or 'storm' occurring during operations on patients with untreated hyperthyroidism. Mechanical stimulation not only of the glandular tissue but also of the thyroid nerves, reflex stimulation by several kinds of stimuli, and liberation of abnormal amounts of certain hormones in the blood, may all release thyroid hormones.

Thyroid uptake of TSH

The striking vasomotor reactions observed even after relatively weak stimuli seem to support the view that the thyroid blood flow is regulated by a mechanism unlike that of many other vascular areas. This is a very old view, supported by findings of contractile endothelial cushions in small arteries at their branchings (HORNE, 1892; MODELL, 1933). This delicate mechanism makes is possible for the nervous vasomotor system to regulate blood flow in all the parts of the gland in a uniform way although the tissue undergoes such rapid morphological changes as those described in the thyroid in re-

sponse to TSH. As the uptake of TSH by the cells of the gland apparently is a function of the amount of blood coming in contact with the cells, a precise flow regulation seems to be even more purposeful. Degenerative changes of the valvular mechanism may therefore be an important aetiological factor in nodular goitre.

SUMMARY AND CONCLUSIONS

A technique permitting the continuous and simultaneous measurements of flow rates and radioactive iodine concentrations of blood entering and leaving the thyroid gland was devised and employed in 19 cats and 66 rabbits. The findings obtained constitute a description of short term changes in thyroid blood flow, iodine uptake and hormone secretion in response to various stimuli; because of their rapidity these reactions have largely escaped previous experimental studies.

The thyroid was dissected free of its surrounding, its largest vein was cannulated and the others ligated. Each drop of blood which issued from the cannula was counted and fell on a moving strip of filter paper. The interval between the appearance of successive drops was measured and recorded by an electronic ordinate writer. The drops on the filter paper were dried and then individually analyzed for radioiodine. A similar analysis of arterial blood was made. This provided the simultaneous descriptions of blood flow and arteriovenous concentration differences, and these two factors then permitted the calculation of the rate of uptake of iodine and the rate of secretion of hormonal iodine.

Thyroid blood flow responded rapidly to nervous and hormonal stimuli. Various degrees of vasoconstriction and vasodilatation were seen. Nervous stimulation produced greater changes than physiological concentrations of hormones circulating in the blood. Alterations of systemic blood pressure and circulating blood volume were reflected with considerable amplification by fluctuations in thyroid blood flow, especially if the gland was large and the resting flow high. Anaesthetics were only slightly effective in influencing thyroid blood flow. Various drugs exerted both direct and reflex changes.

Rate of iodine uptake was in most experiments largely proportional to blood flow. Arteriovenous concentration differences of radioiodine

had a small range of variation within physiological rates of flow. The A–V difference decreased during vasoconstriction and increased during vasodilatation. Adrenaline produced vasoconstriction and concomitant diminution in iodine uptake but pretreatment with large doses of thyrotropic hormone (TSH) disturbed this simple relation and produced increases in uptake which were unrelated to blood flow. It was not possible to predict from the rate of flow whether trapped iodine was utilized by the gland or not. Rate of uptake and rate of blood flow varied from animal to animal.

Secretion of labelled thyroid hormone was not related to blood flow but rather followed the administration of exogenous TSH; other data suggest a similar dependence upon the titre of endogenous TSH. Nervous stimulation, acetylcholine and serotonin augmented secretory activity in the thyroid only in the presence of TSH-induced secretion. Similarly, adrenaline and noradrenaline in small doses also increased thyroid secretion but these hormones were inhibitory in larger doses. Nervous stimuli were more potent in altering secretion rate than were physiological amounts of circulating hormones other than TSH. The response to most of these stimuli had a rather short latency. Rate of secretion was certainly independent of blood oxygen tension and, to a large extent, of anaesthetic agents.

From the measurements of blood flow, uptake of iodine and secretion of hormone it is concluded that rate of secretion and rate of uptake are not parallel in the acute experiment. This was verified by simultaneous measurements of uptake and secretion with two radioactive isotopes of iodine that could be distinguished because of their different half-lives.

The validity of the experimental technique and the physiological significance of the results are discussed. Thyroid activity may be a useful index of vegetative changes both in physiological and pharmacological experiments, even in animals subjected to small operations under anaesthesia. The method of direct measurement of the arteriovenous concentration difference and blood flow that has been presented, measures changes in the thyroid with considerable accuracy and good temporal resolution. It may be used *in vivo* not only for estimations of iodine metabolism in the thyroid but also for studies of other organs whose venous outflow can be properly recorded.

ACKNOWLEDGEMENTS

It is a great pleasure for me to express my sincere gratitude to Professor RAGNAR GRANIT, Director of the Nobel Institute for Neurophysiology, for encouragement, advice and criticism.

I am particularly indebted to Dr. CURT VON EULER, my friend and teacher, for many years of guidance and collaboration. He generously placed instruments and his laboratory at my disposal for part of this investigation, and he has always been ready to share his great knowledge of physiology.

I am deeply grateful to Dr. BERNARD WALTMAN for his kindness in reading the manuscript and for his able criticism and valuable suggestions.

Finally, my deep gratitude is due to all the members of the Institute who have given me great help, and in particular to Mrs. EVI REIGO who prepared the illustrations. Valuable technical assistance has been performed by Miss ANNE-MARIE BENGTSON, Mrs. INGER EGNELL-HOLMGREN, Miss EWA ENGSTRÖM, ULLA HEDENSTEDT, GUNNEL JOHANSSON och Miss GUNVOR LARSSON. Miss LARSSON also typed the manuscript.

I^{132} was obtained by the courtesy of Dr. LARS-GUNNAR LARSSON and Dr. JERZY EINHORN, Radiumhemmet, Karolinska Sjukhuset, Stockholm.

Serotonin and derivatives of lysergic acid were kindly supplied by SANDOZ A. G., Basel; Thyrotropic hormone in large quantities and ACTH by A. B. FERRING, Malmö; Ritalin and DOCA by CIBA PRODUKTER A. B., Stockholm; and Matinol by ERCO PHARMACEUTICALS, Stockholm.

The research reported in this document has been sponsored by the Office of Scientific Research of the *Air Research* and *Development Command, United States Air Force,* through its European Office, under Contract AF 61 (514)–1083.

REFERENCES

ABRAHAMS, V. C. and PICKFORD, M., J .Physiol. 1954. *126.* 329.

ACKERMAN, N. B., and ARONS, W. L., Fed. Proc. 1956. *15.* 1.

ALBRIGHT, E. C., LARSON, F. C. and DEISS, W. P., J. clin. Invest. 1955. *34.* 44.

ARMIN, J., GRANT, R. T., PELS, H. and REEVE, E. B., J. Physiol. 1952. *116.* 59.

BACQ, Z. M., II[e] Congr. Int. Biochemie, 1952. 59.

BACQ, Z. M. and GHIRETTI, F., Arch. int. Physiol. 1951. *59.* 288.

BARBOUR, H. G., Res. Publ. Ass. nerv. ment. Dis. 1940. *20.* 449.

BLAKE, W. D., Amer. J. Physiol. 1957. *192.* 393.

BORELL, U., Acta med. scand. 1945. Suppl. 161.

BORELL, U. and HOLMGREN, HJ., Acta endocr., Copenhagen 1949. *3.* 331.

BOTTARI, P. M., Ciba Foundation Colloquia on Endocrinology 1957, *11.* 52.

BOTTARI, P. and MARTINI, L., Int. J. appl. Radiation and Isotopes 1958. *3.* 81.

BRECHER, G. A., *Venous Return.* Grune & Stratton, New York. 1956

BROWN-GRANT, K., Ciba Foundation Colloquia on Endocrinology 1957. *10.* 97.

BROWN-GRANT, K., VON EULER, C., HARRIS, G. W. and REICHLIN, S., J. Physiol. 1954. *126.* 1.

BROWN-GRANT, K. and GIBSON, J. G., J. Physiol. 1955. *127.* 328.

BURN, J. H., TRUELOVE, L. H. and BURN, I., Brit. med. J. 1945. *1.* 403.

CHESNEY, A. M., CLAWSON, T. A. and WEBSTER, B. Johns Hopk. Hosp. Bull. 1928. *43.* 261.

CHILDS, D. S. JR., KEATING, R. F. JR., RALL, J. E., WILLIAMS, M. M. D. and POWER, M. H., J. clin. Invest. 1950. *29.* 726.

COOK, G. B., EAKINS, J. and VEALL, N., Int. J. appl. Radiation and Isotopes 1956. *1.* 85.
COPE, C. L., Quart. J. Med. 1938. *7.* 151.
COURRIER, R., Ciba Foundation Colloquia on Endocrinology 1957. *10.* 21.
D'ANGELO, S. A., Brookhaven Symp. Biol. 1955. *7.* 9.
D'ANGELO, S. A. and GORDON, A. S., Endocrinolygy 1950. *46.* 39.
D'ANGELO, S. A., PASCHKIS, K. E., GORDON, A. S. and CANTAROW, A., J. clin. Endocr. 1951. *11.* 1237.
DANOWSKI, T. S., JOHNSTON, S. Y. and GREENMAN, J. H., J. clin. Endocr. 1950. *10.* 519.
DIETRICH, S. and SCHWIEGK, H., Arch. exp. Path. Pharmak. 1932. *165.* 53.
DOWLING, J. T., FREINKEL, N. and INGBAR, S. H., J. clin. Endocr. 1956. *16.* 1491.
DUNCAN, D. L., J. Physiol. 1954. *125.* 475.
EISLER, P., Anat. Anz. 1900. *17.* 183.
ERSPARMER, V., Pharmacol. Rev. 1954. *6.* 425.
VON EULER, C., Acta physiol. scand. 1947. *14.* Suppl. 45.
VON EULER, C., J. cell. comp. Physiol. 1950. *36.* 333.
VON EULER, C. and HOLMGREN, B., J. Physiol. 1956 *a. 131.* 125.
VON EULER C. and HOLMGREN, B., J. Physiol. 1956 *b. 131.* 137.
VON EULER, C. and SÖDERBERG, U., EEG Clin. Neurophysiol. 1957. *9.* 391.
VON EULER, U. S., Pharmacol. Rev. 1954. *6.* 15.
VON EULER, U. S. and FOLKOW, B., Arch. exp. Path. Pharmak. 1953. *219.* 242.
FOLKOW, B., STRÖM, G. and UVNÄS, B., Acta physiol. scand. 1949. *17.* 317.
GALLI-MAININI, C., Endocrinology 1942. *30.* 166.
GROSS, J. and PITT-RIVERS, R., Lancet 1952. *1.* 439.
GROSS, J. and PITT-RIVERS, R., Biochem. J. 1953. *53.* 652.
GROSSMAN, M. I., MOELLER, H. C. and PALM, L., Proc. Soc. exp. Biol., N. Y. 1955. *90.* 106.
GROSSMAN, M. I., PALM, L., BECKER, G. H. and MOELLER, H. C., Proc. Soc. exp. Biol., N. Y. 1954. *87.* 312.
GRUNDY, H. F. and HOWARTH, F., J. Physiol. 1957. *138.* 215.

GUNNING, R. E. L., Amer. J. Physiol. 1917. *44.* 215.
GYLLENSTEN, L., Acta Anat. 1953. *17.* Suppl. 18.
HAMILTON, J. G. and LAWRENCE, J. H., J. clin. Invest. 1942. *21.* 624.
HAMILTON, L. H. and LOWENTHAL, J., Endocrinology 1956. *58.* 546.
HARRIS, G. W. and WOODS, J. W., Ciba Foundation Colloquia on Endocrinology 1957. *10.* 3.
HÄUSLER, P., Wien. med. Wschr. 1951. *101.* 557.
HEIDENHAIN, M., Z. wiss. Mikr. 1915, *32.* 361.
HEKTOEN, L., CARLSON, A. J. and SCHULHOF, R., Amer. J. Physiol. 1927. *81.* 661.
HENRY, J. P., GAUER, O. H., and REEVES, J. L., Circulation Res. 1956. *4.* 85.
HERTZ, S. and OASTLER, E. G., Endocrinology 1936. *20.* 520.
HORNE, R. M., Lancet 1892. *2.* 1213.
HORST, W. and RÖSLER, H., Klin. Wschr. 1953. *31.* 13.
INGVAR, D. H., Acta physiol. scand. 1955. *33.* 169.
INGVAR, D. H. and SÖDERBERG, U., EEG Clin Neurophysiol. 1956. *8.* 403.
INGVAR, D. H. and SÖDERBERG, U. Acta physiol. scand. 1958. In press.
KOPSCH, Fr., *Rauber-Kopsch. Lehrbuch und Atlas der Anatomie des Menschen.* 15. Aufl. Thieme, Leipzig. 1939.
LAMBERG, B.-A., Acta med. scand. 1953. Suppl. 279.
LANDGREN, S., Acta physiol. scand. 1952. *26.* 35.
LARSSON, L.-G., Acta radiol. Stockh. 1955. Suppl. 126 .
LEVEY, H. A. and SOLOMON, D. H., Endocrinology 1957. *60.* 118.
LEVINE, H., REMINGTON, R. E.. and VON KOLNITZ, H., J. Nutr. 1933. *6.* 347.
LINDGREN, P., Acta physiol. scand. 1955. *35.* Suppl. 121.
LUDFORD, R. J. and CRAMER, W., Proc. Roy. Soc., B. 1928. *104.* 28.
LUNDBERG, A., Acta physiol. scand. 1952. *26.* 252.
LUNDBERG, A., Physiol. Rev. 1958. *38.* 21.
MC KINNA, J. A. and MISIEWICS, J. J., J. Physiol 1954. *124.* 44P.
MAGNUS, R. and SCHÄFER, E. A., J. Physiol. 1901. *27.* 9.
MAGOUN, H. W., HARRISON, F., BROBECK, J. R. and RANSON, S. W., J. Neurophysiol. 1938. *1.* 101.
MANLEY, O. T., J. Amer. med. Ass. 1916. *67.* 260.

MANLEY, O. T. and MARINE, D., Proc. Soc. exp. Biol., N. Y. 1915. *12.* 202.
MARAZZI, A. S. and HART, E. R. Communication included in PAGE, I. H. and MCCUBBIN, J. W., Circulation Res. 1953. *1.* 354.
MASON, A. S., Memoirs of the Society for Endocrinology 1953. *1.* 31.
MEANS, J. H., The Thyroid and its Diseases. 2nd ed. Lippincott, Philadelphia. 1948.
MODELL, W., Anat. Rec. 1933. *55.* 251.
MYANT, N. B., J. Physiol. 1953. *120.* 278.
NONIDEZ, J. F., Arch. Neurol. Psychiat., Chicago 1931. *25.* 1175.
NONIDEZ, J. F., Amer. J. Anat. 1931. *48.* 299.
NONIDEZ, J. F., Amer. J. Anat. 1932. *49.* 479.
NONIDEZ, J. F., Amer. J. Anat. 1935. *57.* 135.
NONIDEZ, J. F., Amer. J. Anat. 1935. *57.* 259.
OSSOKIN, N., Z. Biol. 1914. *63.* 443.
PAGE, I. H., Physiol. Rev. 1954. *34.* 563.
PALME, F., Z. ges. exp. Med. 1943. *113.* 514.
PAPPENHEIMER, J. R., Physiol. Rev. 1953. *33.* 387.
PETERS, J. P., MAN, E. B. and HEINEMANN, M., Yale J. biol. Med. 1948. *20.* 449.
PICHOTKA, J., Beitr. path. Anat., 1953. *113.* 169.
POCHIN, E. E., Lancet 1950. *259.* 41. 84.
PURPURA, D., Amer. J. Physiol. 1956. *186.* 250.
PURVES, H. D. and GRIESBACH, W. E., Brit. J. exp. Path. 1949. *30.* 23.
RAPELA, C. E. and HOUSSAY, B. A., Rev. Soc. argent. Biol. 1952. *28.* 7.
RAWSON, R. W., STERNE, G. D. and AUB, J. C., Trans. Amer. Ass. Study Goiter 1941. 159.
REICHLIN, S. and REID, A. A., Proc. Soc. exp. Biol., N. Y. 1955. *89.* 212.
REID, G. and RAND, M., Aust. J. exp. Biol. med. Sci. 1951. *29.* 401.
REIN, H., LIEBERMEISTER, K. and SCHNEIDER, D., Klin. Wschr. 1932. *11.* 1636.
RIGGS, D. S., Pharmacol. Rev. 1952. *4.* 284.
RIGGS, D. S., LAVIETES, P. H. and MAN,E. B., J. biol. Chem. 1942. *143.* 363.
RING, C. C., Amer. J. Physiol. 1939. *125.* 244.

ROBBINS, J. and RALL, J. E., Recent Progr. Hormone Res. 1957. *13.* 161.
ROBERTS, S., Ciba Foundation Colloquia on Endocrinology 1957. *11.* 167.
ROCHE, J., LISSITZKY,S. and MICHEL, R., C. R. Acad. Sci., Paris 1952. *232.* 2047.
ROTHLIN, E., J. Pharm. 1957. *9.* 569.
SCHNEIDER, P., *Topographische Anatomie des Vorderhalses beim Kaninchen und der Kehlkopf desselben.* Diss. Berlin. 1867.
SEIDELL, A. and FENGER, F., J. biol. Chem. 1913. *13.* 517.
SHIPLEY, R. A. and MACINTYRE, F. H., J. clin. Endocr. 1954. *14.* 309.
SIMKIN, B., STARR, P. and HANCOCK, C., J. clin. Endocr. 1952. *12.* 940.
SONENBERG, M. and MONEY, W. L., Recent Progr. Hormone Res. 1955. *11.* 43.
SPENCER, A. W., St. Barth. Hosp. Rep. 1934. *67.* 201.
SPITZER, J. J. and MILLER, H. I., Proc. Soc. exp. Biol., N. Y. 1956. *92.* 124.
STANBURY, J. B., BROWNELL, G. L., RIGGS, D. S., PERINETTI, H., DEL CASTILLO, E. and ITOIZ, J., J. clin. Endocr. 1952. *12.* 191.
STANBURY J. B. and HEDGE, A. N., J. clin. Endocr. 1950. *10.* 1471.
TAUROG, A., WHEAT, J. D. and CHAIKOFF, I. L., Trans. Amer. Goiter Ass. 1955. 228.
TRAUBE, L., Zbl. med. Wiss. 1865. *3.* 881.
TROXLER, F. and HOFMANN, A., Helv. chim. acta. 1957. *40.* 1706.
UDENFRIEND, S., SHORE, P. A., BOGDANSKI, D. F., WEISSBACH, H. and BRODIE, B. B., Recent Progr. Hormone Res. 1957. *13.* 1.
UOTILA, U. U., Res. Publ. Ass. nerv. ment. Dis. 1940. *20.* 580.
VERNEY, E. B., Proc. Roy. Soc., B. 1947. *135.* 25.
WASE, A. W. and FOSTER, W. C., Proc. Soc. exp. Biol. N. Y. 1956. *91.* 89.
WATTS, C. F., Amer. J. Physiol. 1915. *38.* 356.
WIDÉN, L., Acta physiol. scan. 1955. *33.* Suppl. 117.
WYNGAARDEN, J. B., WRIGHT, B. M. and WAYS, P., Endocrinology 1952. *50.* 537.

APPENDIX

The physiology and anatomy of thyroid nerves and blood vessels are poorly reviewed in text-books and monographs. Below follows a list which, although far from complete, makes it possible for the reader to find most of the literature of this particular field. Modern descriptions of morphological changes in the thyroid in response to TSH have been excluded. They can easily be found in reviews of the bioassay of TSH. Discussions of the clinical use of sympathectomy at the beginning of this century have also largely been excluded from the list.

A. *General anatomy and anatomy of thyroid blood vessels*

(References to the early literature in ROLLESTON, *The Endocrine Organs in Health and Disease*, Oxford, 1936.)

MURRAY, *Description Arteriarum Corporis Humani.* Upsalie. 1798.
KING, Guy's Hosp. Rep. 1836. *1.* 429.
KOHLRAUSCH, Müller's Arch. 1853.
BARKOW, *Die Blutgefässe, vorzüglich die Schlagadern des Menschen in ihren minder bekannten Bahnen und Verzweigungen.* Breslau. 1866.
SCHNEIDER, 1867 (reference list).
BABER, Philos. Trans. 1876.
ZEISS, *Mikroskopische Untersuchungen über den Bau der Schilddrüse.* Strassburger Dissertation. 1877.
WÖLFLER, *Bau und Entwicklung der Schilddrüse.* Berlin. 1880.
BABER, Philos. Trans. 1881.
JAEGER-LUROTH, *Die Regio thyroidea mit besonderer Berücksichtigung der Blutgefässe.* Strassburger Dissertation. 1883.
BIONDI, Berl. klin. Wschr. 1888. Nov. 19.
LANGENDORFF, Arch. Anat. Physiol., Lpz. 1889. Suppl.
LUSTIG, Arch. ital. Biol. 1891.
HORNE, 1892 (reference list).
ANDERSSON, His' Arch. Anat. 1894. 177.

HÜRTHLE, Pflüg. Arch. ges. Physiol. 1894. *56.*
SCHMIDT, Virchows Arch. 1894. *137.* 330.
KOHN, Arch. mikr. Anat. 1895.
KOHN, Arch. mikr. Anat. 1896.
BÉRARD, *Contribution à l'anatomie et à la chirurgie du goître.* Thèse. Lyon. 1896.
STREIF, Arch. mikr. Anat. 1896. *48.*
CYON, *Die Gefässdrüsen als regulatorische Schutzorgan des Zentralnervensystems.* Berlin *1910.*
THANE, *Quain's Elements of Anatomy. Vol. 2. Part. 2.* 1899.
COCO, Anat. Anz. 1901. Feb. 23.
FLINT, Johns Hopk. Hosp. Bull. 1903.
TSCHEUWSKY, Pflüg. Arch. ges. Physiol. 1903. 97. 280.
REGAUD and PETITJEAN, Bibliographie Anatomique 1905. *14.*
LANDSTRÖM, *Ueber Morbus Basedowii. Eine Chirurgische und Anatomische Studie.* Diss. Stockholm 1907.
MAJOR, Amer. J. Anat. 1909. *9.* 475.
HESSELBERG, Frank. Z. Path. 1910. *5.* 322.
MANLEY and MARINE, 1915 (reference list).
BENSLEY, Amer. J. Anat. 1916. *19.* 37.
MANLEY, 1916 (reference list).
RABL, Arch. mikr. Anat. 1922. *96.* 256.
SHIGYO, Fukuoka Ikwa Daigaku Zasshi 1923. *16.* 326.
WILSON, Anat. Rec. 1927. *37.* 31.
MARINE, *The thyroids, parathyroids, and thymus. I. The thyroid. Special cytology,* E. V. Cowdy ed. New York, 1928. *1. Sect. 17.* 551.
WANGENSTEEN, Surg. Gynec. Obstet. 1929. *48.* 613.
WILSON, Anat. Rec. 1929. *42.* 243.
KURKOWSKY, Z. Anat. Entwick. 1930. *91.* 692.
WILLIAMS, Anat. Rec. 1941. *79.* 263.
KEATING, RAWSON, PEACOCK and EVANS, Endocrinology 1945. *36.* 137.
TURNER and TURNER,Endocrinology, 1945. *36.* 32.
DEMPSEY, Ann. N. Y. Acad. Sci. 1949. *50.* 336.
DEMPSEY, GREEP and DEANE, Endrocrinology 1949. *44.* 88.
DE ROBERTIS, Ann. N. Y. Acad. Sci. 1949. *50.* 317.
GERSH, J. Endocr. 1950. *6.* 282.
PICHOTKA, Arch. exp. Path. Pharmak. 1952. *215.* 299.
PICHOTKA, Arch. exp. Path. Pharmak. 1952. *215.* 317.
PICHOTKA, Arch. exp. Path. Pharmak. 1952. *216.* 268.
BRAUNSTEINER, FELLINGER and PAKESCH, Endocrinology 1953. *53.* 123.
MONROE, Anat. Rec. 1953. *116.* 345.
PICHOTKA, VON KÜGELGEN and DAMANN, Arch. exp. Path. Pharmak. 1953. *220.* 398.
DEL CONTE and STUX, Nature 1954. *173.* 83.

B. *Anatomy of thyroid nerves and morphological effects of stimulation and of denervation*

KÖLLIKER, Handb. Gewebelehre Mensch. 2. Aufl. 1855.
POINCARÉ, J. Anat., Paris 1875.
HORSLEY, Lancet 1886. *2.* 1163.
ANDERSSON, 1894 (list A, "General Anatomy").
KATZENSTEIN, Arch. Anat Physiol., Lpz. 1897. 235.
MISSEROLI, Arch. Fisiol. 1908–1909. *6.* 582.
RHINEHART, Amer. J. Anat. 1912. *13.* 91.
OSSOKIN, 1914 (reference list).
CANNON, BINGER and FITZ, Amer. J. Physiol. 1915. *36.* 363.
MANLEY, and MARINE, 1915 (reference list).
MANLEY, 1916 (reference list).
CANNON and CATTELL, Proc. nat. Acad. Sci., Wash. 1916. *2.* 319.
BRAEUCKER, Klin. Wschr. 1923. *2.* 1074.
BRAEUCKER, Anat. Anz. 1923. *56.* 225.
SCHILF and HEINRICH, Dtsch. med. Wschr. 1924. *50.* 1756.
KIJONO, Virchows Arch. 1925. *257.* 430.
CRAWFORD and HARTLEY, J. exp. Med. 1925. *42.* 179.
NONIDEZ, 1931–1935 (reference list).
VOGT, Arch. exp. Path. Pharmak. 1931. *162.* 129.
VOGT, Arch. exp. Path. Pharmak. 1931. *162.* 197.
NAKAOKA, Ber. ges. Physiol. 1934. *78.* 115.
SUNDER-PLASSMANN, Klin. Wschr. 1934. *13.* 364.
FLORENTIN. FONTAINE and HENNEQUIN, C. R. Soc. Biol., Paris 1936. *121.* 1410.
REID and HOLMAN, Arch. Surg. 1936. *32.* 452.
MEYER, Zbl. inn. Med. 1937. *58.* 209.
ROSS and MOORHOUSE, Quart. J. exp. Physiol. 1938. *27.* 209.
RING, 1939 (reference list).
UOTILA, Endocrinology 1939. *25.* 63.
BROCK, DOTY, KRASNO and IVY, Endrocrinology 1940. *27.* 504.
LOWE, IVY and BROCK, Endocrinology 1945. *36.* 130.
HOLMGREN and NAUMANN, Acta endocr., Copenhagen 1949. *3.* 215.
LAZORTHES, *Le système neurovasculaire.* Masson et Cie, Paris, 1949.
SCHARRER, Verh. anat. Ges., Jena 1953. *51.* 5.

C. *Thyroid blood flow (observation and measurements)*

KING, 1836 (list A, "General Anatomy").
MEULI, Pflüg. Arch. ges. Physiol. 1884. *33.* 378.
MEULI-HILTY, Pflüg. Arch. ges. Physiol. 1886. *38.* 339.

SACERDOTTI, Int. Mschr. Anat. Physiol. 1894. *11.* 6.
BRIAU, *L'innervation du corps thyroide.* Paris. 1898.
CYON, Pflüg. Arch. ges. Physiol. 1898. *70.* 126.
LÜBCKE, Virchows Arch. 1902. *167.* 490.
TSCHEUWSKY, Pflüg, Arch. ges. Physiol. 1903. *97.* 210.
SINAKEWITSCH, Arch. int. Physiolol. 1906. *4.* 51.
FRANÇOIS-FRANCK, J. Physiol. Path. gén. 1908. *10.* 442.
SINAKEWITSCH, *Zur Frage der vasomotorischen Nerven der Schilddrüse.* Diss. 1908.
OSSOKIN, 1914 (reference list).
RAHE. ROGERS, FAWCETT and BEEBE, Amer. J. Physiol. 1914. *34.*. 72.
WATTS, 1915 (reference list).
GUNNING, 1917 (reference list).
HUNT, Amer. J. Physiol. 1918. *45.* 197.
SCHKAWERE and KOTSCHGERIN, Z. exp. Path. Ther. 1925. *45.* 142.
ENGEL, Pflüg. Arch. ges. Physiol. 1926. *211.* 433.
HICKS, J. Physiol., 1926–1927. *62.* 198.
MASON, MARKOWITZ and MANN, Amer. J. Physiol. 1930. *94.* 125.
STURM, Z. ges. exp. Med. 1930. *74.* 514.
VOGT, 1931 (list B, "Nerves").
DIETRICH and SCHWIEGK, 1932 (reference list).
REIN, LIEBERMEISTER and SCHNEIDER, 1932 (reference list).
WILKINS and EICHNA, Johns Hopk. Hosp. Bull. 1941. *68.* 512.
POCHIN, 1950 (reference list).
ROCHE, MICHEL, MICHEL, DELTOUR and LISSITZKY, Biochim. biophys. acta 1951. *6.* 572.
LACY, ORVIS and ALBERT, Endocrinology 1955. *57.* 482.
SHINABERGER and BRUNER, Fed. Proc. 1956. *15.* 483.

D. *Adrenaline and sympathetic influence on thyroid activity*

BRIAU, 1898 (list C, "Blood Flow").
LÜBCKE, 1902 (list C, "Blood Flow").
WIENER, Arch. exp. Path. Pharmak. 1909. *61.* 297.
ASHER and FLACK, Z. Biol. 1911. *55.* 83.
ASHER and VON RODT, Zbl. Physiol. 1912. *26.* 223.
RAHE, ROGERS, FAWCETT and BEEBE, 1914 (list C, "Blood Flow").
CANNON, BINGER and FITZ, 1915 (list B, "Nerves").
WATTS, 1915 (reference list).
CANNON and CATTELL, 1916 (list B, "Nerves").
CANNON and FITZ, Amer. J. Physiol. 1916. *40.* 126.
BURGET, Amer. J. Physiol. 1917. *44.* 492.
MARINE, ROGOTT and STEWART, Amer. J. Physiol. 1918. *45.* 268.
ROGOFF, J. Pharmacol. 1918. *12.* 193.

CANNON and SMITH, Proc. Soc. exp. Biol., N. Y. 1920 *16*. 88.
VON DYKE, Amer. J. Physiol. 1921. *56*. 168.
SCHILF and HEINRICH, 1924 (list B, "Nerves").
HICKS, 1926–1927 (list C, "Blood Flow").
ASHER and PFLÜGER, Klin. Wschr. 1927. *6*. 1614.
HEKTOEN, CARLSON and SCHULHOF, 1927 (reference list).
ASHER and PFLÜGER, Z. Biol. 1928. *87*. 115.
HANCE and HANEY, Amer. J. Physiol. 1932. *102*. 249.
HANEY, Amer. J. Physiol. 1932. *101*. 49.
LEE and BACQ, Amer. J. Physiol. 1933. *103*. 637.
REISS, *Die Hormonforschung und ihre Methoden*. Urban & Schwarzenberg, Berlin u. Wien. 1934.
TRENDELENBURG, *Die Hormone*. Bd. 2. Springer, Berlin. 1934.
HASAMA, Pflüg. Arch. ges. Physiol. 1936. *238*. 758.
ROSSINE, KRITCHEVSKAIA and SEMENOV, Amer. J. Physiol. 1936. *97*. 631.
FRIEDGOOD and BEVIN, Amer. J. Physiol. 1939. *125*. 153.
UOTILA, 1939 (list B, "Nerves").
FRIEDGOOD and CANNON, Endocrinology 1940. *26*. 142.
BROCK, DOTY, KRASNO and IVY, 1940 (list B, "Nerves").
ASHER and RUETSCH, Z. Biol. 1940. *100*. 1.
HELIN and ZILLIACUS, Acta physiol. scand. 1941. *1*. 317.
LOWE, IVY and BROCK, 1945, (list B, "Nerves").
SOFFER, GABRILOVE and JAILER, Proc. Soc. exp. Biol., N. Y. 1949. *71*. 117.
WILLIAMS, JAFFE and KEMP, Amer. J. Physiol. 1949. *159*. 291.
BOTKIN and TEW, Proc. Soc. exp. Biol., N. Y. 1952. *79*. 378.
BOTKIN and JENSEN, Endocrinology 1952. *50*. 68.
D'ANGELO, Fed. Proc. 1956. *15*. 44.
BROWN-GRANT and GIBSON, J. Physiol. 1956. *131*. 85.

7376